Sleep as a marker of cortical plasticity during development

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Maya Ringli-Grünenwald

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Prof. Dr. Lutz Jäncke and Prof. Dr. Reto Huber

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Summary

From the beginning of sleep research the question about its function has dominated many studies: Why do we sleep? Up to now, no conclusive answer has been found. However, careful reflection has led to the perspective of sleep serving more than one function at the same time. In the last ten years, the synaptic homeostasis hypothesis has taken a great impact on sleep research (Tononi and Cirelli, 2003, 2006). This model postulates a relationship between sleep and synaptic plasticity. According to the hypothesis plastic processes occurring during wakefulness produce a net increase in synaptic strength, which is followed by higher energy consumption and spatial extension and gradually leads to a saturation of our learning capacity. The role of sleep is to reduce synaptic strength to a baseline level, which is sustainable in terms of energy and space requirements. At the same time this downscaling process has a beneficial effect on learning and memory.

Regarding behaviour, childhood and adolescence is a phase of essential learning processes. These processes are accompanied by substantial structural and physiological changes in the brain. At the same time sleep undergoes major changes. Thus, the aim of the present thesis was to investigate the interaction between sleep, brain maturation and behaviour during development.

The thesis starts with an overview about the relationship between slow wave activity (SWA, power in the electroencephalogram (EEG) between 1-4.5Hz) during deep sleep and cortical plasticity during brain maturation (Research Part I). Several studies in humans and animals as well as in a computer model indicate that synaptic changes are reflected in SWA of the surface EEG, such that more or stronger synapses are correlated to higher SWA (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007; Vyazovskiy et al., 2008; Vyazovskiy et al., 2009). Synapse density follows the timecourse of an inverted U-shaped time course, showing a strong increase during childhood and a decline during adolescence (Huttenlocher and Dabholkar, 1997). Strikingly, at the same time SWA exhibits a similar

pattern (Feinberg, 1982). This leads to the questions how these two factors are related: Does SWA merely reflect cortical maturation or does sleep play an active role in brain development? A model was established to discuss this question (Ringli and Huber, 2011). It suggests that, in childhood, during wakefulness more synapses are built or strengthened than are eliminated during the night. This leads to a progressive increase of synaptic density. During adolescence the opposite is true, which results in a total reduction of synaptic density. Finally during adulthood both processes are balanced, leading to a stable number of synapses (Huttenlocher, 1979). In summary, the model describes a way of how downscaling during slow wave sleep may actively contribute to the elimination of synapse and therefore impact cortical maturation as a whole.

The first study of research part II investigates the relationship between SWA and cortical maturation by means of magnetic resonance imaging (MRI). Changes in SWA of children and adolescents were compared to the decline in grey matter volumes across the brain. For the first time a direct relationship between grey matter and SWA was shown, even independent of age. Specifically, grey matter volumes in areas correlating positively with SWA largely overlapped with brain regions showing a strong developmental decline of grey matter at that time (Buchmann et al., 2010).

In the second study local aspects of sleep are investigated. Children between 2 and 19 years were recorded by the use of high-density EEG (hd-EEG, 128 channels) to study regional differences. In accordance with earlier studies in adults (Werth et al., 1996; Cajochen et al., 1999; Finelli et al., 2001b) also children exhibited regional differences in the distribution of SWA: SWA was highest over occipital areas in the youngest children, while in later years the local maxima shifted over central parts during childhood to frontal regions in late adolescence (Kurth et al., 2010a). This time course parallels cortical (Shaw et al., 2008) and functional maturation (Luna and Sweeney, 2004). Based on numerous studies reporting a close relationship between SWA and plastic changes (Huber et al., 2004; Huber et al., 2006; Tononi and Cirelli, 2006; Huber et al., 2007b; Landsness et al., 2009) it can be concluded that SWA may serve as a

marker of cortical maturation. Additionally, SWA topography might serve as a useful tool to uncover synaptic changes.

Finally this tool is applied in two specific groups in research part III. In a first study, the SWA topography was compared between boys and girls. The analysis showed that girls exhibited higher SWA in bilateral language regions, while in boys SWA was increased in a frontal area involved in spatial processing (Ringli et al., submitted-a). In line with the studies observing that sex differences seem to be most pronounced regarding language skills and spatial abilities, on the structural as well as on the behavioural level, these findings indicate that SWA mirrors use-dependent differences of specific skills. In the second study SWA topography was applied in a group of children diagnosed with attention-deficit/hyperactivity disorder (ADHD). Recent studies revealed a delay in grey matter maturation in ADHD patients (Shaw et al., 2006b; Shaw et al., 2007). The analysis of the SWA topography in ADHD children showed a shift of the local maximum in the posterior direction (Ringli et al., submitted-b). In accordance to the finding that the local maximum reflects the stage of brain maturation (Kurth et al., 2010a), the result may be interpreted as neuromaturational delay in children with ADHD.

In summary, these studies confirm a close relationship between synaptic plasticity in the course of brain maturation as well as in respect of use-dependent functional differences. The analysis of SWA topography, considered by hd-EEG, serves as a sensitive tool to uncover developmental and functional changes in healthy and pathological processes.

Zusammenfassung

Seit dem Beginn von wissenschaftlichen Untersuchungen des Schlafvorgangs war die Frage nach dessen Funktion in vielen Studien vorherrschend: Warum schlafen wir? Eine schlüssige Antwort ist bis heute nicht gefunden. Wohl aber hat ein Umdenken stattgefunden, so dass heute in der Schlafforschung weniger nach der *einen* Funktion des Schlafs gesucht wird, sondern vielmehr verschiedene Prozesse nebeneinander vermutet werden. In den letzten zehn Jahren hat die Synaptische Homöostase Hypothese einen wichtigen Stellenwert in der Schlafforschung eingenommen (Tononi and Cirelli, 2003, 2006). Sie postuliert für den Zusammenhang zwischen Schlaf und synaptischer Plastizität ein Modell, welches besagt, dass plastische Prozesse im Wachzustand zu einer Verstärkung von Synapsen in neuronalen Schaltkreisen führt. Dies wirkt sich auf den Energieverbrauch sowie den Platzbedarf aus, was schliesslich unsere Lernkapazitäten mehr und mehr einschränkt. Die Aufgabe des Schlafvorgangs ist nun gemäss der Hypothese die stufenweise Reduktion von synaptischen Stärken, zurück auf ein Ausgangsniveau welches energetisch sowie im Bezug auf die räumliche Ausdehnung tragbar ist. Gleichzeitig hat dieser Vorgang nützliche Folgen für Lern- und Gedächtnisvorgänge.

Die Zeitspanne von Kindheit und Adoleszenz ist eine Phase grosser Lernvorgänge auf der Verhaltensebene, die im Gehirn von erheblichen Umbau- und Anpassungsprozessen begleitet werden. Gleichzeitig vollziehen sich auch im Schlaf markante Veränderungen. Die Untersuchung dieser wechselseitigen Beziehungen zwischen Schlaf, Gehirnentwicklung und Verhalten ist das Ziel dieser Doktorarbeit.

Als erstes erfolgt eine Übersicht über den Zusammenhang der langsamwelligen Aktivität (SWA, vom Englischen *slow wave activity,* Aktivität im Elektronzephalogramm (EEG) zwischen 1-4.5Hz) im Tiefschlaf und kortikaler Plastizität während der Entwicklung. Verschiedene Studien am Menschen sowie am Tier- und Computermodell haben gezeigt, dass Veränderungen der

synaptischen Stärke direkt im Oberflächen-EEG reflektiert werden (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007; Vyazovskiy et al., 2008; Vyazovskiy et al., 2009): Stärkere oder dichtere Synapsen korrelieren demnach mit grösserer SWA. Die Synapsendichte verläuft während der Entwicklung in einer umgekehrt U-förmigen Kurve, d.h. mit einem steilen Anstieg während der Kindheit gefolgt von einer deutlichen Abnahme während der Adoleszenz (Huttenlocher and Dabholkar, 1997). Interessanterweise folgt zeitgleich auch die SWA diesem Verlauf (Feinberg, 1982). Es stellt sich die Frage nach der Interaktion der beiden Faktoren: Wird die Gehirnreifung in der SWA nur reflektiert oder spielt der Schlaf eine aktive Rolle im Bezug auf die Entwicklung? Die Frage wird anhand eines Modells diskutiert (Ringli and Huber, 2011): Dieses postuliert, dass während der Kindheit tagsüber mehr Synapsen gebildet bzw. verstärkt als nachts eliminiert werden, was eine Zunahme der synaptischen Dichte zur Folge hat. Der umgekehrte Vorgang findet während der Adoleszenz statt, was gesamthaft eine Synapsenreduktion zur Folge hat. Im Erwachsenenstatus sind schliesslich beide Prozesse ausbalanciert (Huttenlocher, 1979). Das Modell beschreibt insgesamt eine Möglichkeit, wie der Schlaf anhand des Reduktionsvorgangs während des Tiefschlafs aktiv zur Eliminierung von Synapsen und daher generell zur Reifung des Gehirns beitragen könnte.

In der zweiten Arbeit wird der Zusammenhang von SWA und kortikaler Maturierung mittels Kernspintomographie genauer untersucht. Die SWA von Kindern und Jugendlichen werden mit der Abnahme der grauen Substanz in verschiedenen Gehirnregionen verglichen. Die Studie bestätigt damit zum ersten Mal den direkten Zusammenhang zwischen grauer Substanz und SWA, unabhängig vom Alter. Bemerkenswerterweise ist dieser Zusammenhang in jenen Gehirnarealen am stärksten ausgeprägt, in denen zeitgleich die markantesten anatomischen Veränderungen stattfinden (Buchmann et al., 2010).

Die dritte Publikation widmet sich regionalen Aspekten von Schlaf. Mit hochaufgelöstem EEG (hd-EEG, vom Englischen *high density EEG*), bestehend aus 128 Elektroden, werden lokale Unterschiede in

der SWA in Kindern zwischen 2 und 19 Jahren untersucht. In Übereinstimmung mit früheren Studien bei Erwachsenen (Werth et al., 1996; Cajochen et al., 1999; Finelli et al., 2001b) bestätigt sich, dass auch bei Kindern die SWA nicht gleichmässig verteilt ist, sondern lokale Unterschiede zeigt: Die SWA war bei den jüngsten Kindern über okzipitalen Gebieten am stärksten ausgeprägt und zeigte im Laufe der Kindheit eine Verschiebung des lokalen Maximums über zentrale Regionen bis hin zum Frontalkortex in der späten Adoleszenz (Kurth et al., 2010a). Dieser Verlauf entspricht der anatomischen (Shaw et al., 2008) wie auch der funktionalen Entwicklung (Luna and Sweeney, 2004). Basierend auf zahlreichen Studien, die einen engen Zusammenhang zwischen SWA und plastischen Veränderungen nachgewiesen haben (Huber et al., 2004; Huber et al., 2006; Tononi and Cirelli, 2006; Huber et al., 2007b; Landsness et al., 2009), deuten diese Befunde darauf hin, dass topographische Unterschiede in der SWA während der Entwicklung Prozesse der kortikalen Maturierung widerspiegeln. SWA kann demzufolge als ein Indikator für Gehirnreifung betrachtet werden. Zudem wird die Darstellung der SWA Topographie als Instrument zur Erfassung von plastischen Veränderungen vorgeschlagen.

Dieses Instrument wird in den zwei letzten Arbeiten angewendet. Als erstes wurde die Topographie von Mädchen und Knaben verglichen. Die Analyse ergab höhere SWA in bilateralen Sprachgebieten bei den Mädchen, während die Knaben mehr SWA in einer in räumliche Verarbeitung involvierte Region des Frontalkortex zeigten (Ringli et al., submitted-a). Im Kontext der Literatur über geschlechtstypische Struktur- sowie Leistungsunterschiede, die am stärksten im Bereich der Sprache sowie der räumlichen Wahrnehmung beobachtet wurden, sprechen diese Befunde dafür, dass sich der unterschiedliche Gebrauch von Funktionen in der SWA widerspiegelt. Als nächstes wird das Instrument bei Kindern mit Aufmerksamkeitsdefizit/Hyperaktivitätsstörung (ADHS) angewendet. Jüngste Längsschnitt-Studien sprechen dafür, dass bei diesen Kindern die Gehirnreifung im Vergleich zu gesunden Kindern verzögert ist (Shaw et al., 2006b; Shaw et al., 2007). Die Analyse der SWA-Topographie ergab, dass Kinder mit ADHS im Vergleich zu gesunden Kindern eine räumliche Verschiebung des SWA Maximum

nach posterior aufwiesen (Ringli et al., submitted-b). Dieser Befund lässt sich vor dem Hintergrund, dass das lokale SWA-Maximum jeweils über der zeitgleich am stärksten reifenden Gehirnregion liegt, als Indikator für eine verzögerte neuronale Entwicklung bei Kindern mit ADHD interpretieren.

Zusammenfassend bekräftigen diese Arbeiten einen engen Zusammenhang zwischen synaptischen Veränderungen, sowohl während der kortikalen Entwicklung als auch im Bezug auf funktionale Unterschiede. Die Anwendung von hd-EEG mit der Analyse der SWA-Topographie eignet sich folglich als Instrument zur Erfassung von entwicklungsbedingten sowie auch funktionalen Veränderungen im Kontext von gesunden wie auch pathologischen Prozessen.

1

Introduction

In recent years, the body of literature emphasizing the importance of sleep for efficient daytime functioning is increasing. In particular, there is growing evidence suggesting that sleep is directly involved in cortical plasticity (Sejnowski and Destexhe, 2000; Steriade and Timofeev, 2003; Born et al., 2006; Tononi and Cirelli, 2006), a feature necessary for proper development during childhood and adolescence as well as for efficient adapting to changes in everyday life. Specifically, the low-frequency high-amplitude waves during non-rapid eye movement (NREM) sleep, an electrophysiological marker of sleep depth (Blake and Gerard, 1937), seem to play a key role in cortical plasticity.

While in the beginnings sleep was mainly investigated on the basis of its behavioural characteristics (e.g. typical body posture or increased arousal threshold), sleep research has entered a new era after the discovery of the electroencephalography (Berger, 1929), which enabled the continuous recording of neuronal activity on the surface of the scalp.

The recording and analysis of the sleep EEG

The analysis of the sleep electroencephalogram (EEG) is based on standard procedures. Sleep stages are defined (scored) on the basis of the sleep EEG, muscle tone activity, measured by the Electromyogram (EMG) and eye movements, recorded by the Electrooculogram (EOG). The sleep EEG is derived from electrodes on the scalp. For the registration of eye activity electrodes are placed above and below the outer canthus, muscle activity is measured with mental or submental electrodes.

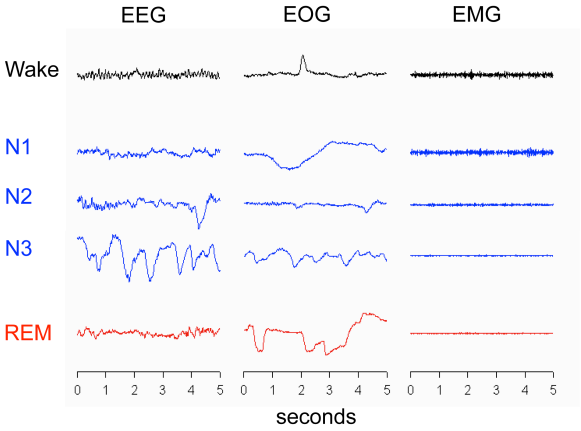

Figure 1. *Different vigilance stages measured by EEG, EOG and EMG. W (wake), N1 (NREM sleep stage 1), N2 (NREM sleep stage 2), N3 (NREM sleep stage 3), REM sleep.*

To provide reliability between researchers a standardized scoring manual was made available for the first time by Alan Rechtschaffen and Anthony Kales in 1968 (Rechtschaffen and Kales, 1968) and was revised recently by the American Academy of Sleep Medicine (AASM) (Iber et al., 2007). Based on frequency, amplitude and waveform of the EEG (defined in epochs of 20 or 30 seconds) sleep can be distinguished from wakefulness and further differentiated into rapid-eye-movement (REM) sleep or three stages of non-rapid eye movement (NREM) sleep (Figure 1). During wakefulness alpha activity (8-13Hz) is covering more than 50% of the epoch and is most prominent over the occipital region, while muscle tone is of variable

amplitude and eye movements are clearly detectable (blinks, reading movements, irregular conjugate rapid eye movements). Usually, the slow, sinusoidal eye movements indicate the transit to NREM sleep stage 1 with the alpha rhythm (less than 50%) being replaced by a low amplitude, predominantly 4-7Hz activity and vertex sharp waves. After a few minutes in sleep stage 1 (N1), people typically progress to stage 2 (N2), which is defined by the presence of K complexes and sleep spindles, followed, especially at the beginning of the night, by a period comprised of stage 3 (N3). In Stage 3, slow waves of 0.5-2Hz and a peak-to-peak amplitude >75μV are the most prominent characteristics, occurring in more than 20% of an epoch. While eye movements are usually not seen in stage 2 and 3 and EMG is of variable amplitude, irregular, sharply peaked eye movement and low muscle tone, accompanied by low amplitude, mixed frequency EEG are clear indicators of REM sleep. In the course of a sleep episode the two sleep states, NREM sleep and REM sleep alternate in a cyclic manner, each sleep cycle lasting between 90 and 120 minutes in adults (Aserinsky and Kleitman, 1953) (Figure 2). During development, the normal cycle length is shorter, lasting approximately 50 to 110 minutes in preschool children (Bes et al., 1991) and 50 to 60 minutes during early infancy (Jenni et al., 2004). Slow wave sleep is prominent early in the night, especially during the first sleep cycle, and diminishes as the night progresses. As slow wave sleep wanes, periods of REM sleep lengthen.

While sleep stage scoring may vary in accordance to the rater (Stanley, 1996) power spectral analysis represents an objective analysis of the sleep EEG. In the present thesis spectral analysis was performed by Fast Fourier Transformation (FFT,(Cooley and Tukey, 1965)). In this mathematical algorithm, the time-based EEG is transformed into the frequency domain, by decomposing the signal into sine and cosine functions of varying amplitude and frequency (Geering et al., 1993). This procedure results in a power density spectrum which is expressed as power per bandwidth. The frequency resolution is determined by the length of the analyzed epoch (e.g. 4s for a resolution of 0.25Hz). A smoothed power spectrum can be obtained by averaging over several consecutive epochs and over frequency bins.

The use of large number of scalp electrodes as is the case with high-density EEG (hd-EEG, 128 channels, Figure 3), enables higher spatial resolution which offers the possibility to analyze variations in local distribution of spectral power. A graphical display is used to present such data by means of colour coded topographical maps, where regional EEG power differences become apparent. Thereby, the values are plotted on the planar projection of a scalp model and interpolated between the electrodes.

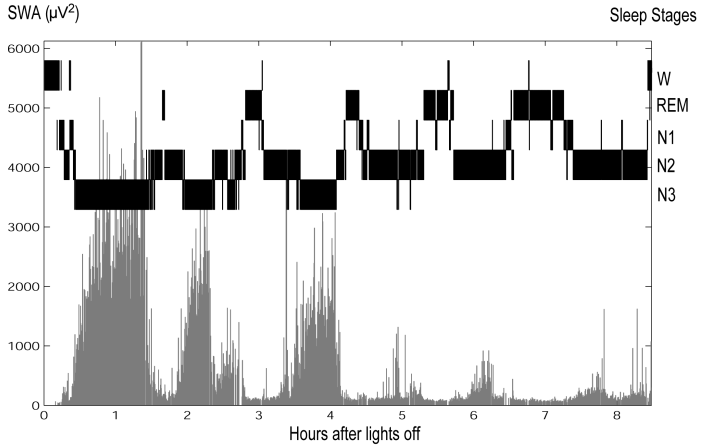

Figure 2. *Hypnogram and time course SWA in a 11 years old boy. Sleep stages are indicated in black bars. SWA (grey bars) in μV^2 is maximal at the beginning of the sleep period and decreases across the night.*

Neurophysiology of the sleep EEG

During NREM sleep the transition from the low-voltage, fast activity EEG observed during wakefulness to the characteristic EEG of slow wave sleep is due to the occurrence of depolarized up states and periods of hyperpolarized down states, in thalamocortical and cortical neurons (Steriade et al., 1993; Timofeev et al., 2001). The alternation between episodes of synchronized firing and states of complete neuronal silence, with the frequency of about 1 Hz, is termed slow oscillation. Intracellular recordings have shown that during NREM sleep compared to REM sleep or wakefulness, virtually every cortical

neuron engages in the slow oscillation (Steriade et al., 1993; Amzica and Steriade, 1998; Timofeev et al., 2001). The repeated occurrence of down states characterized by synaptic silence is probably the reason why brain metabolism and blood flow are diffusely reduced during NREM sleep as compared to wakefulness (Braun et al., 1997). Moreover, a close temporal relationship between these cellular phenomena and simultaneously recorded slow waves on the surface was shown, such that down states correspond to the negative part of the surface slow waves and up states to the positive part (Amzica and Steriade, 1998; Vyazovskiy et al., 2009).

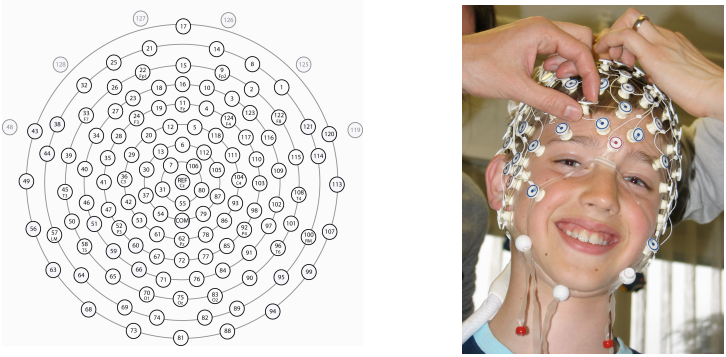

Figure 3. *Left: 128-Channel Map. Right: HydroCel™ Geodesic Sensor Net.*

Mechanisms of sleep regulation

It was discovered early on that arousal thresholds – measured for example as the duration of an acoustic stimulus required to awaken a sleeping subject – is positively correlated with the amount of slow-waves in the EEG of NREM sleep (Blake and Gerard, 1937). In the same study, it was further noticed that high amplitude slow waves predominate in the first two hours of sleep and decrease thereafter. Later it was shown that the amount of slow wave sleep is positively correlated with the duration of prior waking (Webb and Agnew, 1971), suggesting that this aspect of sleep is homeostatically regulated.

In 1982 Alexander Borbély proposed the two-process model of sleep regulation, which postulates that sleep propensity is determined by the interaction of a homeostatic process S and a circadian process C (Borbely, 1982). Process S increases during waking and decreases during sleep. An important advance has been the demonstration that Process S is reflected accurately by the amount of slow wave activity (SWA, EEG power between 1 and 4.5 Hz) during NREM sleep (Borbely, 1982; Borbély and Achermann, 2005). Therefore the positive relationship between slow waves and the duration of wakefulness is best seen under the influence of sleep deprivation. If we are not allowed to sleep and are forced to stay awake longer than usual, sleep pressure mounts and soon becomes overwhelming. The more we stay awake, the longer and more intensely we sleep afterwards: arousal thresholds increase, there are fewer awakenings. As repeatedly shown in both humans and mammals, SWA increases exponentially with the duration of prior wakefulness and decreases exponentially during sleep, therefore reflecting the accumulation of sleep pressure during wakefulness and its release during sleep. Thus, sleep is homeostatically regulated. While the immediate history of sleep and waking determines the level of Process S, process C is independent of prior sleep and waking because it is generated by an intrinsic pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus. Process C is thought to modulate the timing of sleep episodes by enforcing an upper and a lower threshold so that whenever one of these thresholds is reached by process S a sleep episode is terminated or initiated.

Two important aspects of the model have been revealed by the use of special study designs. First, in sleep deprivation protocols it was shown that sleep loss can be recovered by an intensification of slow wave sleep, reflected in an increase of SWA, and not necessarily by an increase in sleep duration (Borbély and Achermann, 2005). Second, the independence of the homeostatic from the circadian process was confirmed by SCN-lesioned rats. These animals no longer exhibit circadian modulation of sleep and wakefulness. Nevertheless, sleep deprivation still results in an increase of SWA (Mistlberger et al., 1983; Tobler et al., 1983; Trachsel et al., 1992).

Interestingly at birth the compensatory increase in SWA is not yet present. Human and animal neonates react to a selective or total sleep deprivation with an increase in NREM sleep duration only (Anders and Roffwarg, 1973; Bes et al., 1991; Thomas et al., 1996; Frank et al., 1998; Jenni et al., 2004). Once the dynamics of sleep homeostasis according to the two-process model have been established, the build-up of homeostatic sleep pressure during wakefulness is faster in both pre-pubertal children and rats compared to young adolescents or post-pubertal rats respectively (Alfoldi et al., 1990; Jenni et al., 2005). Still, not only sleep homeostasis but also other characteristics of sleep undergo various alterations in the course of development.

Sleep across age

Childhood is typically characterized by a decrease in total sleep length (Iglowstein et al., 2003). Moreover, in sleep architecture the proportion of NREM and REM sleep changes through the reduction of REM sleep (Roffwarg et al., 1966b; Jenni and Carskadon, 2007). Substantial changes are also observed in NREM sleep: The activity of slow waves follows the time course of an inverted U-shaped curve (Feinberg, 1982; Gaudreau et al., 2001; Jenni and Carskadon, 2004; Campbell and Feinberg, 2009). SWA increases during childhood, reaches maximal size shortly before puberty and decreases during adolescence (Feinberg et al., 2006; Campbell and Feinberg, 2009).

Because development is a phase of substantial changes in brain morphology and function (Johnson, 2001) and slow waves originate from synchronized activity of cortical neurons (Steriade et al., 1993; Vyazovskiy et al., 2009), it can be assumed that brain maturation, which results in remarkable cortical reorganization, should be reflected in the sleep EEG. In fact, longitudinal and cross-sectional studies confirm that age-dependent changes of the sleep EEG are most pronounced in the slow wave frequency band (Jenni et al., 2004; Jenni and Carskadon, 2004; Feinberg et al., 2006; Kurth et al., 2010b).

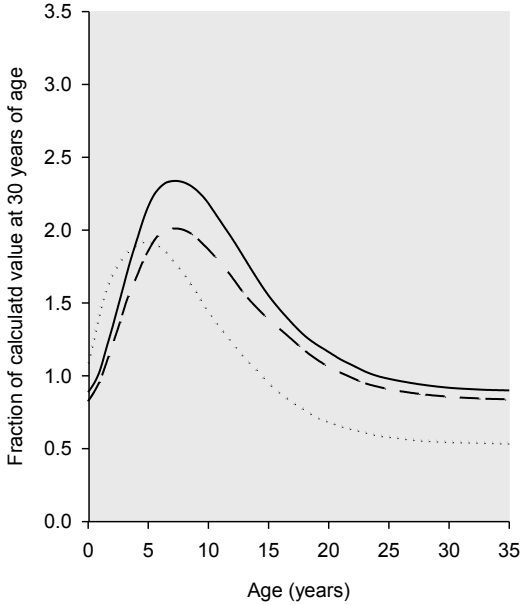

Figure 4. *Gamma distribution model describing the time course of synaptic density (Farbe), cerebral metabolic rate (Farbe) and delta wave amplitude (Farbe) across age. (adapted from Feinberg and Campbell, 2010)*

Sleep and plasticity

Already in 1982 Feinberg alluded to the parallel time course of slow wave amplitude and synaptic density, proposing that the decrease of SWA during adolescence may reflect the decrease of synapses through pruning (Feinberg, 1982; Campbell and Feinberg, 2009; Feinberg and Campbell, 2010). (Figure 4) This proposition became more conceptional in light of a recently formulated, comprehensive hypothesis, the synaptic homeostasis hypothesis (Tononi and Cirelli, 2006).

The synaptic homeostasis hypothesis

The synaptic homeostasis hypothesis provides a model for the relationship between SWA and synaptic plasticity (Figure 5). During

wakefulness, we interact with the environment and acquire information about it. The neuromodulatory milieu (for example, a high level of noradrenaline, NA; (Cirelli and Tononi, 2004) favors the storage of information, which occurs largely through synaptic potentiation (Trachtenberg et al., 2002). A key functional corollary of the hypothesis is that plastic changes during wakefulness have a cost in terms of energy requirements, space requirements, supplies of key cellular constituents, and progressively saturates our capacity to learn. When we go to sleep, we become virtually disconnected from the environment (Steriade et al., 1993). Changes in neuromodulatory milieu when falling asleep trigger slow oscillations (Steriade and Timofeev, 2003). The changed neuromodulatory milieu (e.g. low NA; (Cirelli and Tononi, 2004)) also ensures that synaptic activity is not followed by synaptic potentiation, which makes adaptive sense given that synaptic activity during sleep is not driven by interactions with the environment. Since the average strength of synaptic connections at the end of the wake period has increased, neurons are more easily synchronized and the resulting slow waves of early sleep are of high amplitude (Esser et al., 2007; Vyazovskiy et al., 2009). The slow oscillations, however, are not just an epiphenomenon of increased synaptic strength, but according to the hypothesis have a role to play. Specifically, the repeated sequences of depolarization – hyperpolarization of slow oscillations would lead to the proportional downscaling of all synapses impinging on each neuron (Turrigiano and Nelson, 2000, 2004). This results in an overall decrease of synaptic strength and therefore in benefits in terms of energy and space requirements and, due to increased signal-to-noise ratios, also in terms of learning and memory (Olcese et al., 2010). Thus, when we wake up, neural circuits do preserve a trace of previous experiences, but are kept efficient at a recalibrated level of synaptic strength.

The close relationship between sleep SWA and synaptic strength was shown in various species using key markers of synaptic strength: In cortical slices the frequency and amplitude of miniature excitatory post-synaptic currents (Liu et al., 2010), in Drosophila melanogaster the protein levels of key components of central synapses (Bushey et al., 2011), in rats the slope of the local field potential evoked by

electrical cortical stimulation (Vyazovskiy et al., 2008) and in humans the slope of transcranial magnetic stimulation evoked EEG responses (Bellina et al., 2008), all increased after wakefulness and decreased during sleep.

Synaptic plasticity is an important key feature of cortical maturation as well as of learning processes. In the next sections the relationship between sleep SWA and these two aspects is introduced.

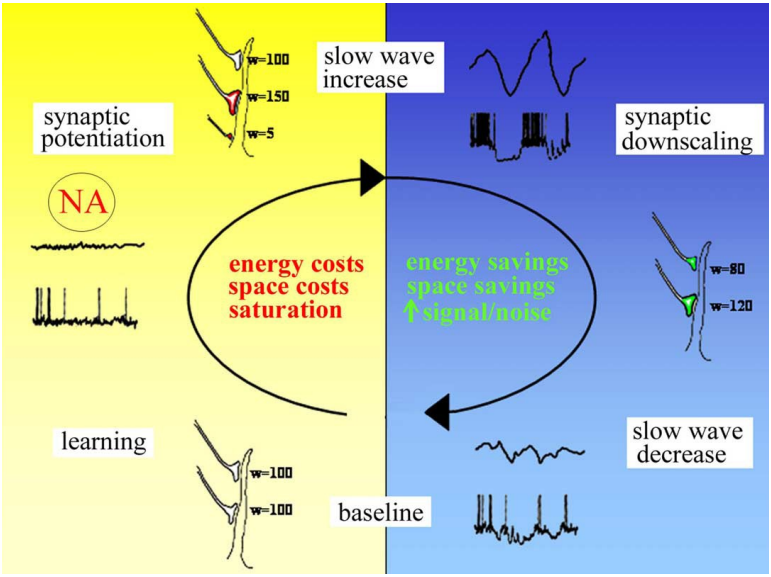

Figure 5. *The synaptic homeostasis hypothesis. (Source: Tononi and Cirelli, 2006)*

SWA and cortical maturation

During early childhood neurons grow bushier and establish more numerous connections to other cells (DeFelipe, 1997). Moreover, axons initially explore areas much wider than their final targets (Gao et al., 1999). Then, in the course of adolescence, more synapses are eliminated than formed (Zuo et al., 2005b), in part through activity-dependent processes (Hua and Smith, 2004). Synaptic pruning during adolescence is accompanied by a reorganization of neuronal

connections whereby mistargeted axons and unused synapses are eliminated, and connectivity becomes more specific. The decrease of synaptic density during adolescence, which is reflected in changes in grey matter, proceeds asynchronously in different brain areas (Paus, 2005), in line with the maturation of specific skills (Luna and Sweeney, 2004; Shaw et al., 2006a).

Changes in synaptic density are paralleled by changes in slow wave amplitude (Huttenlocher, 1979; Feinberg, 1982; Huttenlocher and Dabholkar, 1997) and brain metabolism, presumably due to the increased energy requirements associated with increased synaptic activity (Chugani, 1998) (Figure 4). This observation has been confirmed both in humans and in rats (Nakamura et al., 1999; Glantz et al., 2007). As suggested by the synaptic homeostasis hypothesis (Tononi and Cirelli, 2006), and confirmed by computer simulations and experimental studies in both humans and rats, changes in synaptic efficacy can account for the observed changes in sleep slow waves (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007; Vyazovskiy et al., 2009; Olcese et al., 2010). Thus, sleep SWA could be taken as a reliable indicator of net changes in average synaptic density/strength both in the course of the night (sleep homeostasis) and in the course of development.

Because synaptic density can only be investigated postmortem in humans, numerous studies have instead measured changes in grey matter volume with MRI to examine cortical maturation. These studies have reported preadolescent volume increases and subsequent decreases during adolescence across wide parts of the cortex, revealing regional differences in maturation with some areas maturing earlier and others later (Giedd et al., 1999; Gogtay et al., 2004; Sowell et al., 2004; Giedd and Rapoport, 2010).

In summary, the parallel time course of synaptic changes, energy consumption and SWA indicates that plastic changes during cortical maturation are related to changes in sleep SWA.

Use-dependent changes in SWA

Evidence for a link between SWA and plastic changes not only arises from maturational studies but also from settings in which synaptic

changes are triggered experimentally. Recordings obtained with high-density EEG, a tool that enables the detection of power differences with high spatial resolution, show that reduced motor activity due to arm immobilization during the day is followed by a local decrease of SWA over the corresponding motor region compared to a normal night (Huber et al., 2006). On the other hand, potentiation of synapses (with transcranial magnetic stimulation or intense training) leads to a local increase of SWA over the corresponding brain region in adults (Huber et al., 2004; Huber et al., 2007b), as well as in children (Ringli et al., 2009). Together these findings indicate a direct relationship between synaptic strength and SWA. Interestingly, the increase of SWA predicted improvement of performance in a retest after sleep. Later a causal relation of SWA and behaviour was demonstrated by studies in which SWA was suppressed by means of acoustic stimulation: Not only local SWA was diminished (Landsness et al., 2009), also no learning improvement was present after sleep (Aeschbach et al., 2008; Landsness et al., 2009). On the other hand the stimulation of slow oscillation like potential fields by transcranial application of oscillating potentials (0.75 Hz) during the first NREM sleep lead to enhanced improvement in a declarative memory task compared to sham stimulation (Marshall et al., 2006). These findings further support the hypothesis that SWA plays an active role in the regulation of cortical synaptic strength.

Nevertheless, local differences in the distribution of sleep SWA are also found after a normal day without a specific training commonly initiated in experimental studies (Werth et al., 1996; Cajochen et al., 1999; Finelli et al., 2001b), demonstrating a strong predominance of SWA over frontal regions in adults (Werth et al., 1996; Finelli et al., 2001a). Such findings are in line with a use-dependent regulation of SWA, because in adults the frontal cortex is the brain area most “used” or plastic (Horne, 1993; Couyoumdjian et al., 2010).

In summary, there is good evidence that sleep SWA is a reliable indicator of net changes of synaptic strength in the course of a night (sleep homeostasis), which seems directly related to the observed post-sleep performance improvements.

Investigating sleep SWA in populations with behavioural differences

Since SWA seems to reflect functional changes in the course of development as well as in use-dependent behaviour, the investigation of sleep in particular groups, differing in behaviour, could give continuative insight into the involved processes.

Sex differences

In adults numerous functional differences between males and females have been reliably described (Kimura, 2000). It was shown that males and females display differences in certain cognitive skills that are unrelated to differences in the general level of intelligence. The study of sex difference in neuroscience is an important approach because it serves as a model of how structural or physiological differences are related to functional differences. The understanding of the mechanisms underlying sex differences on the neuronal level uncover characteristics of behaviour that would be predicted by this neuronal feature, also independent of sex. Among the most consistent findings it was reported that men exceed in mental rotation and spatial perception, while women perform better in language tasks (Voyer et al., 1995; Kimura, 2000; Kaiser et al., 2009). On the anatomical level overall larger brain volumes in males have been demonstrated, whereas women exhibit larger local grey matter volumes in a number of regions, even when controlling for brain size (Luders et al., 2009). But sex differences in language and spatial skills seem to be present already early during development on both the behavioural, as well as on the anatomical level (Vogel et al., 2003; Plante et al., 2006; Burman et al., 2008; Moore and Johnson, 2008; Quinn and Liben, 2008; Rubia et al., 2010; Tzuriel and Egozi, 2010; Porter et al., 2011).

While some studies assume that these differences are mainly use-dependent (Feng et al., 2007; Tzuriel and Egozi, 2010), other studies have alternatively suggested that they are due to different trajectories in brain development (Lenroot et al., 2007; Porter et al., 2011). Concerning verbal abilities it was for example argued that sex differences during development may occur because language skills are acquired gradually over time: They claim that boys are delayed in

language acquisition which, if the delay is not catched up until the end of the maturation phase, also explains the differences during adulthood (Blanton et al., 2004; Burman et al., 2008).

Studies on sex differences in sleep during development are scarce. Two studies reported no differences in delta power during childhood but a stronger decline in adolescent girls (Campbell et al., 2005; Feinberg et al., 2006), suggesting that developmental trajectories, i.e. earlier synaptic pruning of grey matter in girls (Giedd et al., 1999; Lenroot et al., 2007), may account for these differences.

Early childhood disorders

Disturbed sleep is a common symptom among a wide range of disturbances. However, in general sleep problems are regarded as epiphenomenon of some other causes. Nevertheless, the growing understanding of the close relationship between cortical plasticity and sleep has led to rethinking. For example, the observation that the generation of slow waves share common mechanisms with the generation of spike wave complexes, a typical feature of electrical status epilepticus during slow wave sleep (ESES), has inspired the study of this disorder, addressing slow waves as a contributing factor. Thus the slope of slow waves, a recently introduced marker of the degree of synchronization of the firing of cortical neurons (Riedner et al., 2007; Vyazovskiy et al., 2009), was studied in patients suffering from ESES. In contrast to healthy control children, the slope of slow waves did not decrease in the course of the night (Bölsterli et al. 2011), possibly indicating a disruption of the downscaling process during sleep (Tononi and Cirelli, 2006). Since synaptic downscaling seems to have a large impact on learning processes (Landsness et al., 2009) this discovery would also explain why the disorder is accompanied by a progressive deterioration of cerebral functioning (Tassinari et al., 1977). Interestingly the onset of ESES typically occurs around the age of 4-5 years and lasts for several month or years, but resolve before adulthood without treatment, indicating a link between brain development and incidence of the disorder. In fact deviations from normal brain development are assumed for many psychiatric disorders, with childhood onset schizophrenia (COS), autism and attention-deficit/hyperactivity disorder (ADHD) among the

best studied examples (Shaw et al., 2010). In recent years a change in focus of neuroimaging studies occurred, by analyzing not only absolute measures, like grey matter volume at a certain age, but rather considering the shape of age by size trajectories, to disentangle the connection between brain maturation and disturbed behaviour. Developmental trajectories are believed to be better predictors of functional characteristics (Shaw et al., 2006a; Lenroot et al., 2007) and therefore could be employed to investigate psychopathological disorders (Giedd, 2008). For instance ADHD, the most common neurobehavioural disorder of childhood with a prevalence of 3% and 12% in the school-age population (Faraone et al., 2003) has experienced clarification by the longitudinal study of cortical maturation in affected children. Not only it was shown that cortical thickness trajectory is delayed in these patients (Shaw et al., 2007) but even more clinical improvement was related to a convergence of developmental trajectories toward typical development, whereas persistence of ADHD was accompanied by a progressive divergence away from typical development (Shaw et al., 2006b).

In summary, since there seems to be a close relationship between cortical changes during brain maturation and sleep SWA, disturbed developmental trajectories may also be reflected in SWA. Especially topographical differences may uncover deviant processes. Therefore, children diagnosed with ADHD may be of special interest in studying developmental features of sleep SWA, because they display both aspects, intensive use, i.e. motor hyperactivity, as well as deviations from normal cortical maturation, whose investigation may offer insights into brain functioning and plasticity in this disorder.

Structure of the thesis and specific aims and hypotheses

Since childhood and adolescence are periods of substantial changes in brain morphology and function (Johnson, 2001), the overall aim of this thesis is to investigate the relationship between cortical plasticity and sleep during development. Because of increasing evidence for a close relationship between slow waves and synaptic plasticity (Tononi and Cirelli, 2006), SWA is the main focus of the examination. Given that brain maturation is a local process with temporal differences between brain regions, hd-EEG was chosen to study spatial variations in SWA.

Research Part I

Overview on sleep slow waves

Ringli and Huber (2011). Developmental aspects of sleep slow waves: Linking sleep, brain maturation and behaviour. Progress in Brain Research

The thesis begins with a general overview (book chapter) about sleep slow waves during development. Changes in SWA during childhood and adolescence are presented together with evidence for a relationship between developmental changes and cortical plasticity. In addition, SWA is linked to cognition and behaviour in health and disease. Particular attention is paid to the characteristics of the time course of SWA. Based on the assumptions of the synaptic homeostasis hypothesis (Tononi and Cirelli, 2006; Olcese et al., 2010) we propose a model of how SWA might actively drive cortical maturation by the regulation of synaptic strength during sleep.

This general opening is followed by two specific analyses which investigated the link between sleep EEG and cortical maturation in more detail.

Research Part II

Slow waves and grey matter maturation

Buchmann, Ringli, Kurth, Schärer, Geiger, Jenni and Huber (2010). EEG sleep slow wave activity as a mirror of cortical maturation. Cerebral Cortex

The decrease of SWA during adolescence was proposed to reflect the elimination of synapses through pruning (Campbell and Feinberg, 2009). Since synaptic density can only be studied postmortem in humans, we measure changes in grey matter volume to assess a direct relationship between cortical maturation and sleep SWA. Because of the similar pattern between age-related changes in SWA and synaptic changes (Feinberg and Campbell, 2010) we hypothesize that the developmental decrease in grey matter volume would be correlated to declining SWA in our subjects.

Slow wave topography across age

Kurth, Ringli, Geiger, LeBourgeois, Jenni and Huber (2010). Mapping cortical activity in the first tow decades of life: a high-density sleep electroencephalogram study. Journal of Neuroscience

In the second study the regional variations in the timing of brain maturation are of special interest. MRI studies reported temporal and regional differences in cortical maturation, with an overall trajectory along the posteroanterior axis (Gogtay et al., 2004; Sowell et al., 2004). Since high-density EEG has proved successful in the detection of spatial variations in the power distribution (Huber et al., 2004), we apply this tool in our sample to uncover regional changes in the sleep EEG during development. Because of the close relationship between synaptic changes and SWA we hypothesize that maturational changes in the sleep EEG would be most pronounced in the slow wave band and reflect age-related regional differences that would resemble the pattern known from MRI studies.

The usefulness of SWA topography as a tool to uncover changes in synaptic plasticity is next tested in two further studies, addressing the question if it is sensitive enough to detect local differences in subjects of the same age but with clear distinctive behaviours.

Research Part III

SWA topography and sex differences

Ringli, Kurth, Jenni and Huber. (2010) The sleep EEG topography in adolescents shows differences in language areas. Submitted.

A close functional relationship between sleep slow waves and synaptic strength has been confirmed in numerous experimental studies (e.g. (Huber et al., 2004). Since it is generally accepted that behavioural and anatomical sex differences exist (Kimura, 2000), starting already during childhood (e.g. (Moore and Johnson, 2008; Ozcaliskan and Goldin-Meadow, 2010), we ask whether these sexually dimorphic characteristics are reflected in the topography of sleep SWA. We hypothesize that cortical regions associated with skills in which one sex outperforms the other, would express more SWA.

SWA topography and ADHD

Ringli, Souissi, Kurth, Brandeis, Jenni and Huber (2010). Topography in sleep slow wave activity in children with attention-deficit/hyperactivity disorder. Submitted

In the last study, the focus changes from health to disease. We apply hd-EEG in a group of children diagnosed with ADHD. Recently longitudinal MRI studies revealed that in children with ADHD the onset of grey matter maturation is delayed by around 3 years (Shaw et al., 2007). Since SWA topography had been established as a marker of brain maturation (Kurth et al., 2010a), we hypothesized that the topography of children with ADHD would reveal a pattern with the local maximum in SWA being shifted to the posterior direction.

Research Part I: General Overview

2

Developmental aspects of sleep slow waves: Linking sleep, brain maturation and behaviour

Maya Ringli[1] and Reto Huber[1,2]

1) Child Development Center, Children's University Hospital Zurich, 8032 Zurich, Switzerland

2) Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland

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Slow Brain Oscillations of Sleep, Resting State and Vigilance

ABSTRACT

Sleep slow waves are the major electrophysiological features of non-rapid eye movement (NREM) sleep. Although there is growing understanding of where slow waves originate and how they are generated during sleep, the function of slow waves is still largely unclear. A recently proposed hypothesis relates slow waves to the homeostatic regulation of synaptic plasticity. While several studies confirm a correlation between experimentally triggered synaptic changes and slow wave activity (SWA) little is known about its association to synaptic changes occurring during cortical maturation. Interestingly slow waves undergo remarkable changes during development that parallel the time course of cortical maturation. In a recent cross-sectional study including children and adolescents the topographical distribution of SWA was analyzed with high-density EEG. The results showed age dependent differences in SWA topography: SWA was highest over posterior regions during early childhood and then shifted over central derivations to the frontal cortex in late adolescence. This trajectory of SWA topography matches the course of cortical grey maturation. In this chapter the major changes in slow waves during development are highlighted and linked to cortical maturation and behaviour. Interestingly synaptic density and slow wave amplitude increase during childhood are highest shortly before puberty, decline thereafter during adolescence, reaching overall stable levels during adulthood. The question arises whether SWA is merely reflecting cortical changes or if it plays an active role in brain maturation. We thereby propose a model, by which sleep slow waves may contribute to cortical maturation. We hypothesize that while there is a balance between synaptic strengthening and synaptic downscaling in adults, the balance of strengthening/formation and weakening/elimination is tilted during development.

INTRODUCTION

On the neuronal level slow (<1Hz) oscillations are the major electrophysiological features of deep non-rapid eye movement (NREM) sleep (Steriade et al., 1993) (Figure 1). When such slow oscillations are synchronized and involve the majority of cortical neurons in a certain brain area, they become visible in the surface EEG as slow waves (Vyazovskiy et al., 2009) (Figure 1).

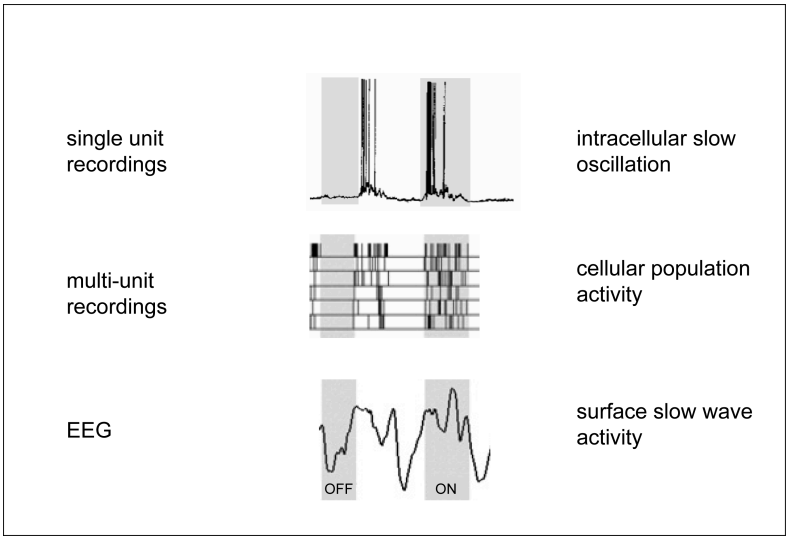

Figure 1: Neuronal activity measured at three different levels. *Top row: Intracellular slow (~0.3-0.4 Hz) depolarizing oscillation measured by single unit recordings (adapted from Steriade et al., 1993). Middle row: Raster plots of neuronal activity in one representative rat showing highly synchronized cellular population activity in early NREM sleep and the corresponding surface EEG showing slow wave activity (bottom row) (adapted from Vyazovskiy et al., 2009).*

Single unit recordings reveal intracellular slow oscillations (<1Hz) with the characteristic alternation of depolarized up- and hyperpolarized down-states (Steriade et al. 1993). This activity is highly synchronized across cellular populations during early sleep, as multi-unit recordings show (Vyazovskiy et al., 2009). Periods of neuronal silence correlate with the negative peak of surface slow wave activity, as measured

with EEG, and high population activity is correlated to the positive deflection of SWA. Yellow squares indicate simultaneous occurrence of activity on all three levels.

The activity of these slow waves is traditionally quantified by EEG spectral analysis. Sleep slow wave activity (SWA; EEG power between 0.75 and 4.5 Hz) was shown to be a precise electrophysiological correlate of the homeostatic regulation of sleep. Sleep homeostasis is a well characterized phenomenon with a strong impact on basic and clinical research (Borbely, 1982; Borbely and Achermann, 1999; Borbély and Achermann, 2005). In recent years there is a growing understanding of where slow waves originate and how they are generated during sleep (Vyazovskiy et al., 2009). However, the functions of the regulation of slow waves are still largely unclear. One interesting aspect is that the activity of slow waves undergoes remarkable changes during development (Feinberg, 1982; Jenni and Carskadon, 2004; Feinberg et al., 2006; Campbell and Feinberg, 2009; Kurth et al., 2010a). SWA increases in the first years of life, reaches a maximum before puberty and then declines rapidly during adolescence into adulthood (Feinberg, 1982; Feinberg and Campbell, 2010). The understanding of mechanisms behind such developmental changes in the activity of slow waves may explain the neurophysiological and cellular processes underlying the need for sleep.

In this chapter we will highlight the changes in slow waves during development, linking cortical maturation, sleep and behaviour. Also we propose a hypothesis for the mechanisms possibly driving the inverted U-shaped timecourse of slow waves.

CHARACTERISTICS OF SLOW WAVES

Definition, generation and behaviour of slow waves

During NREM sleep the transition from the low-voltage, fast activity EEG observed during wakefulness to the characteristic EEG of NREM sleep is due to the occurrence of depolarized up states, episodes of sustained firing and brief periods of hyperpolarization with neuronal silence, also called down states, in thalamocortical and

cortical neurons. Down states are due to reduced activating input from ascending cholinergic and other neuromodulatory pathways (for reviews see: (McCormick and Pape, 1990; Steriade et al., 1993; Llinas and Steriade, 2006), which is primarily due to an increase in leakage potassium conductances (McCormick and Pape, 1990).

Intracellular recordings have shown that during NREM sleep compared to REM sleep or wakefulness, virtually every cortical neuron engages in the slow oscillation that consists of alternating periods of sustained firing or neuronal silence respectively (Steriade et al., 1993; Amzica and Steriade, 1998; Steriade et al., 2001) (Figure 1). The repeated occurrence of down states characterized by synaptic silence is probably the reason why brain metabolism and blood flow are diffusely reduced during NREM sleep as compared to wakefulness, as shown by imaging studies (Braun et al., 1997). Moreover, a close temporal relationship between these cellular phenomena and simultaneously recorded slow waves on the surface was shown (e.g. down states correspond to the negative part of the surface slow waves (Amzica and Steriade, 1998; Vyazovskiy et al., 2009b) (Figure 1).

Human EEG recordings using 256 channels have revealed that, in adults, the slow oscillation behaves as a traveling wave that sweeps across a large portion of the cerebral cortex (Massimini et al., 2004). Slow oscillations seem to originate from nearly any region of the scalp and propagate in any direction. Yet most frequently slow oscillations started in frontal areas and propagated in an anteroposterior direction.

Slow waves and sleep homeostasis

It was discovered early on that arousal thresholds – measured for example as the duration of an acoustic stimulus required to awaken a sleeping subject – is positively correlated with the amount of slow-waves in the EEG of NREM sleep. It was also noticed that high amplitude slow waves predominate in the first two hours of sleep and decrease thereafter (Blake and Gerard, 1937). It was later shown that the amount of slow wave sleep is positively correlated with the

duration of prior waking (Webb and Agnew, 1971), suggesting that this aspect of sleep is homeostatically regulated.

In 1982 Alexander Borbély proposed the two-process model of sleep regulation which postulates that sleep propensity is determined by the interaction of a homeostatic process S and a circadian process C (Borbely, 1982). Process S increases during waking and decreases during sleep. Therefore the positive relationship between slow waves and the duration of wakefulness is best seen under the influence of sleep deprivation. If we are not allowed to sleep and are forced to stay awake longer than usual, sleep pressure mounts and soon becomes overwhelming. The more we stay awake, the longer and more intensely we sleep afterwards: arousal thresholds increase, there are fewer awakenings. Thus, sleep is homeostatically regulated. An important advance has been the demonstration that Process S is reflected accurately by the amount of slow wave activity (SWA, electroencephalographic (EEG) power in the low frequency range between 0.5 and 4 Hz) during NREM sleep (Borbely, 1982; Borbély and Achermann, 2005). As repeatedly shown in both humans and mammals, SWA increases exponentially with the duration of prior wakefulness and decreases exponentially during sleep, thus reflecting the accumulation of sleep pressure during wakefulness and its release during sleep. Therefore, the immediate history of sleep and waking determines the level of Process S.

Homeostatic sleep regulation at the cellular level

The accumulation of sleep pressure during wakefulness and its decline during sleep are not only reflected by EEG SWA but can also be observed at the cellular level. It is well known that at the cellular level cortical neuronal firing patterns are characteristically different in NREM sleep compared to both REM sleep and wakefulness (e.g. (Steriade et al., 2001). However, recently it was shown that cortical neuronal firing patterns not only depend on the behavioural state, but also on how long a rat has been awake or asleep (Vyazovskiy et al., 2009). Unit activity recordings in the rat showed that firing rates change as a function of sleep pressure, showing that higher sleep pressure is related to higher firing rates, which progressively

decrease across sleep episodes. The same study found that also synchrony of firing activity is higher under high sleep pressure during early compared to low sleep pressure late sleep. In summary, this study yields evidence for a homeostatic regulation of sleep at the cellular level, by modulating firing rates. Thus, changes in firing patterns are expressed in the typical homeostatic behaviour of the cortical SWA measured in the surface EEG (Figure 1).

Sleep homeostasis during development

At birth, sleep homeostasis is not yet present in both animal and humans but develops in the first months of life (Bes et al., 1991; Jenni et al., 2004). For example, when very young rats (P12) are sleep deprived, they mainly compensate the sleep dept by increasing sleep duration (Frank et al., 1998). However only twelve days later (P24), sleep deprivation results in an increase in sleep SWA, as is the case in adult animals, whereas sleep duration remains constant. Similarly in humans: selective or total sleep deprivation in human neonates lead to compensatory increases in NREM sleep duration only (Anders and Roffwarg, 1973; Thomas et al., 1996). Moreover, it seems that the dynamics of sleep homeostasis according to the two-process model of sleep undergoes developmental changes. It was shown that the build-up of homeostatic sleep pressure during wakefulness is faster in both pre-pubertal children and rats compared with young adolescents or post-pubertal rats respectively (Alfoldi et al., 1990; Jenni et al., 2005). In contrast, the decline of the homeostatic process is similar in both groups. The following sections all refer to a maturational stage where sleep homeostasis is developed.

However, as the example of sleep homeostasis shows, sleep is not a uniform phenomenon across the lifespan. Specifically, slow waves undergo significant changes during development, which the next section will focus on.

DEVELOPMENT OF SLOW WAVES – DISPARITIES IN INFANTS, CHILDREN AND ADOLESCENTS

It is noteworthy that some properties of slow waves, such as the duration of slow wave sleep (Tucker et al., 2007) or the topography of

slow wave activity (Finelli et al., 2001b) vary impressively between subjects, but intraindividually remain stable over time. However, this intraindividual stability is only true after reaching adulthood. During development the characteristics of slow waves undergo prominent changes until they reach a mature stage.

Slow wave amplitude follows an inverted U-shaped time course

Development is a phase of substantial changes in brain morphology and function (Johnson, 2001). Since slow waves originate from synchronized activity of cortical neurons (Steriade et al., 1993; Vyazovskiy et al., 2009b), it is expected that brain maturation, which results in remarkable cortical reorganization, should be reflected in the sleep EEG. In fact, longitudinal and cross-sectional studies point to major age-dependent changes in the slow wave frequency band (Jenni et al., 2004; Jenni and Carskadon, 2004; Feinberg et al., 2006; Kurth et al., 2010b).

Cross-sectional and longitudinal studies show that SWA follows the time course of an inverted U-shaped curve (Feinberg, 1982; Gaudreau et al., 2001; Jenni and Carskadon, 2004; Campbell and Feinberg, 2009; Kurth et al., 2010b). The amplitude of slow waves increases during childhood and is highest shortly before puberty. Then, in the course of adolescence, slow wave amplitude or SWA declines by over 60% between 11 and 16 years. This decline is slowed down at about 17 years (Feinberg et al., 2006; Campbell and Feinberg, 2009). It is worth mentioning that the decline during puberty even exceeds the decrease of SWA observed over the subsequent 50 years of life (Feinberg and Campbell, 2010).

So far little is known about the development of slow waves in animals. Recently SWA was recorded longitudinally in juvenile rats from post-natal day 25 (P25) to P50. Similar to humans the time-course of SWA followed the course of an inverted U-shape with a peak around the rats pubertal stage (Olini et al., 2010). Importantly, changes in the amount of wakefulness did not explain the decline of SWA during adolescence.

Slow wave topography demonstrates regional shifts

While it is well-known that in adults SWA topography typically shows a frontal predominance (Finelli et al., 2001b), only few studies have looked at regional differences in the changes of SWA and other frequency bands during development (Jenni et al., 2005; Tarokh and Carskadon, 2010).

Recently, EEG power topography was investigated in a broad sample of children and adolescents between age 2 and 20 years (Kurth et al., 2010a). All-night sleep EEG was recorded using high-density EEG with 128 electrodes. The analysis of the topographical distribution of the most common frequency bands in children showed that SWA topography undergoes large changes from early childhood to adolescence (Figure 2) while the topography of power in other frequency bands remained largely unchanged. Notably, a striking finding was that SWA exhibited a regional predominance that was characteristic for a certain age range. When the location of maximal SWA was identified across age, the authors found a shift from posterior to anterior regions, reaching frontal derivations during adolescence. The adult frontally predominated pattern of SWA, as found by Finelli et al. (Finelli et al., 2001b), was still not fully present even in late adolescence. In contrast, none of the other frequency ranges exhibited similar age-related alterations in the topographical pattern during development.

These results are in line with imaging studies, showing that cortical maturation follows a posterior-anterior time course, with lower-order primary areas maturing first, followed by higher-order association areas (Gogtay et al., 2004; Sowell et al., 2004).

DEVELOPMENTAL ASPECTS AND THEIR RELATION TO THE FUNCTION OF SLOW WAVES

Several studies show that the amplitude of slow waves, increases during childhood, reaches its maximum shortly before puberty and decreases during adolescence (Gaudreau et al., 2001; Jenni and Carskadon, 2004; Feinberg et al., 2006; Campbell and Feinberg, 2009). A longitudinal study revealed that during childhood and early puberty SWA correlates with age but not with other developing

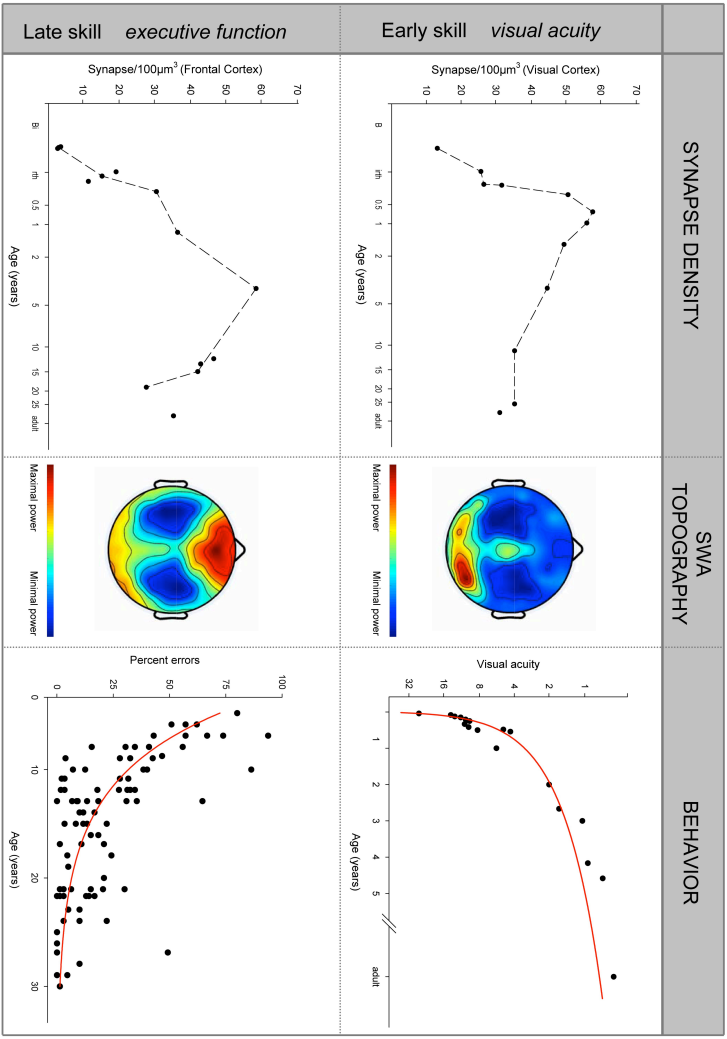

Figure 2: Linking brain maturation, SWA topography and behaviour at an early and a late state of development. *Left column: Mean synaptic density (synapses/100µm3) in visual cortex (area 17) (top) and prefrontal cortex (bottom) at various ages (adapted from Huttenlocher & Dabholkar, 1997). Middle column: Maps of EEG power during NREM sleep (adapted from Kurth et al. 2010). Topographical distribution of NREM sleep SWA for age groups 2-5 years (top) and 17-20 years (bottom). Maps are based on 109*

derivations from the first 60 min of NREM sleep stages 2 and 3. Maps were normalized for each individual and then averaged for each age group. Values are colour coded (maxima in red, minima in blue) and plotted on the planar projection of the hemispheric scalp model. To optimize contrast, each map was proportionally scaled, and values between the electrodes were interpolated. Right column: Development of visual acuity in human infants plotted against age (top) (adapted from Teller 1981). Both axis are logarithmically scaled. Y-axis shows the number of minutes subtended by each black or white stripe of the acuity (smaller number indicating advanced maturation) grating and x-axis age in years. Bottom: Direction error in percentage versus age in the anti-saccade task with the target located on the right side and the correct saccades generated to the left side. The red line represents exponential decline in percent error across age (adapted from Munoz et al. 1998)

Top row: Synaptic density in the visual cortex is highest at around 8 month after birth and decreases thereafter as a matter of maturation, reaching adult levels shortly before puberty (left). Also SWA is highest over the occipital cortex during the first years of life (middle), reflecting gray matter maturation. Brain maturation is accompanied by the specification of skills and behavioural changes. Visual acuity, a function located in the occipital cortex, is developed during the first years of life, reaching adult levels at around 3 years (right; smaller digits indicate better acuity).

Bottom row: An example for a skill maturing at a later state is given at the right. 'Executive functions' is a general term to which a set of cognitive abilities is subsumed. In Munoz et al. (1998) executive functions are tested using the antisaccade task, where subjects are asked to look in the opposite direction of an appearing stimulus. While prepubertal children look at the cue reflexively (which is rated as error), reaction control (suppression of reflexive saccades) is reached during puberty and error rate decreases near 20 years. During the same time, synaptic density in the frontal cortex, where executive functions are located, is starting to decrease, as a sign of brain maturation (left). Paralleling this process SWA is highest over frontal derivations (middle).

biological marker such as weight, height, BMI or sexual maturation (Feinberg et al., 2006). This may be a hint that SWA is possiblyreflecting the driving mechanism underlying brain maturation rather than just being an epiphenomenon of development.

Already in 1982 Feinberg alluded to the similarity of the time course of slow wave amplitude and synaptic density, proposing that the decrease of SWA during adolescence reflects the decrease of synapses through pruning (a process eliminating overproduced synapses which results in an increase of the specification of synaptic connectivity) (Feinberg, 1982; Campbell and Feinberg, 2009; Feinberg and Campbell, 2010). This proposition became more

conceptional in light of a recently formulated, comprehensive hypothesis, the synaptic homeostasis hypothesis (Tononi and Cirelli, 2003, 2006).

The synaptic homeostasis hypothesis

The synaptic homeostasis hypothesis is based on a large number of observations at many different levels, from molecular and cellular biology to systems neurophysiology and neuroimaging (for more details see (Tononi and Cirelli, 2003, 2006).

The main points of the hypothesis are as follows. During wakefulness, we interact with the environment and acquire information about it. The neuromodulatory milieu (for example, a high level of noradrenaline, NA; (Cirelli and Tononi, 2004) favors the storage of information, which occurs largely through synaptic potentiation (Trachtenberg et al., 2002). A key functional corollary of the hypothesis is that, due to the net increase in synaptic strength, such plastic changes during wakefulness have a cost in terms of energy requirements, space requirements, supplies of key cellular constituents, and progressively saturates our capacity to learn. When we go to sleep, we become virtually disconnected from the environment (Steriade et al., 1993). Changes in neuromodulatory milieu when falling asleep trigger slow oscillations (Steriade and Timofeev, 2003). The changed neuromodulatory milieu (e.g. low NA; (Cirelli and Tononi, 2004)) also ensures that synaptic activity is not followed by synaptic potentiation, which makes adaptive sense given that synaptic activity during sleep is not driven by interactions with the environment. Since the average strength of synaptic connections at the end of the wake period has increased, neurons synchronize their firing better and the resulting slow oscillations of early sleep are of high amplitude (Esser et al., 2007; Vyazovskiy et al., 2009b). In the sleep EEG these high amplitude slow oscillations are reflected by increased SWA. The slow oscillations, however, are not just an epiphenomenon of increased synaptic strength, but according to the hypothesis have a role to play. Specifically, the repeated sequences of depolarization – hyperpolarization of slow oscillations would lead to the proportional downscaling of all synapses impinging on each

neuron (Turrigiano and Nelson, 2000, 2004). In other words, the downscaling of synapses leads to an overall decrease of synaptic strength. The reduced synaptic strength reduces the amplitude and synchronization of the slow oscillations, which is reflected in a decrease of SWA in the sleep EEG. Because of the dampening of the slow oscillation, the downscaling process is progressively reduced, making the process self-limiting when synaptic strength reaches a baseline level (Olcese et al., 2010). By returning total synaptic weight to an appropriate baseline level, sleep enforces synaptic homeostasis. Again, the key functional corollary is that synaptic homeostasis has benefits in terms of energy and space requirements, of the supply of key cellular constituents and, due to increased signal-to-noise ratios, in terms of learning and memory (Olcese et al., 2010). Thus, when we wake up, neural circuits do preserve a trace of previous experiences, but are kept efficient at a recalibrated level of synaptic strength, and the cycle can begin again.

In the past years important progress was made in unravelling the originally hypothesized mechanisms. Molecular studies support the idea of a reduction of synaptic strength during the night, by confirming that markers of synaptic potentiation are high after wakefulness and low after sleep, in both rodent cortex/hippocampus and fly brains (Cirelli and Tononi, 2004; Vyazovskiy et al., 2008; Gilestro et al., 2009). Furthermore in slices obtained from frontal cortex of rats and mice it was found that both the frequency and the amplitude of miniature postsynaptic potentials, the most direct reflection of synaptic strength, increase after wakefulness and decreased after sleep (Liu et al., 2010). On the electrophysiological level early findings of single-neuron recordings were extended (Steriade et al., 1993) in that also neuron populations change their firing rate across the night which was closely related to the changes of SWA (Vyazovskiy et al., 2009). High synchrony firing and higher firing rates were found during early sleep and declined across the night. States of hyperpolarization, corresponding to the negative peak slow waves on the surface (Figure 1), were longer and more frequent at the beginning of the night and showed a decrease in incidence and duration in the course of sleep. Alterations in the firing behaviour were highly correlated to the changes in SWA (Vyazovskiy et al.,

2009). Recently recordings from cortical slices provided evidence for alterations of plasticity during sleep. Induction of repetitive burst-pairings in layer V pyramidal cells of the rat was followed by long-term depression, which was inversely related to excitatory postsynaptic potentials, thus suggesting a mechanism by which synaptic inputs are proportionally downsized during NREM sleep (Czarnecki et al., 2007). Furthermore in a computer model the interplay of activity and changes in plasticity was proposed as a regulating mechanism that modulates the renormalization of synaptic strength during NREM sleep (Olcese et al., 2010). The model suggests that the strength of a connection is downregulated by a selflimiting control loop: For example a strong connection leads to high firing rates and synchrony. This will also lead to stronger synaptic depression, which brings the system down to baseline connectivity values. When connections are renormalized activity levels are too low to induce significant plastic changes and the system will reach an equilibrium point.

Synaptic strength is reflected in the slope of sleep slow waves

Wakefulness is associated with a net increase in synaptic strength which is renormalized during sleep (Olcese et al., 2010). The strength of population excitatory postsynaptic currents is reflected by the slope of local field potentials (LFPs) evoked by electrical stimuli (Rall, 1967). Slope and amplitude of LFPs increase as a function of the time spent awake and decrease during sleep (Vyazovskiy et al., 2008). Furthermore the slope of LFPs is positively correlated with the mean and peak SWA of first hour of NREM sleep (Vyazovskiy et al., 2008). Synaptic strength is high at the beginning of the night and most individual neurons start and stop firing in near synchrony with the rest of the population (Vyazovskiy et al., 2009). Synchronous transitions at the unit level were associated with steep slopes of slow waves during early sleep and less synchronous transitions with reduced slopes at the end of the night. Slow wave slope decreased from the beginning to the end of the night as was shown in humans (Riedner et al., 2007), in rats (Vyazovskiy et al., 2007) and in

computo (Esser et al., 2007). The decrease of slope over night was explained as homeostatic reduction of synaptic strength.

This homeostatic regulation, i.e. the reduction of steepness over night is already present during development, as was found by investigating the slope of slow waves in prepubertal children and mature adolescents (Kurth et al., 2010b). Furthermore the comparison of the two groups showed that the slope of children exceeded that of adolescents and remained steeper across the night, in both conditions, during baseline as well as after sleep deprivation. In light of a recently proposed thalamocortical computer model (Esser et al., 2007) these findings might indicate greater synaptic strength of neurons involved in the generation of sleep slow waves in prepubertal children, compared to mature adolescents. Such increased synaptic strength may be due to greater density or greater efficacy of cortical synapses or both.

Since higher synaptic density is related to higher activation during wakefulness an equivalent proportion of downscaling is needed to return to base levels. This observation would explain the parallel time course of synaptic density and slow wave amplitude during development.

In the following part the relationship between cortical maturation and SWA is discussed in more detail.

SWA and cortical maturation

During early childhood neurons grow bushier and establish more numerous connections to other cells (DeFelipe, 1997). Moreover, axons initially explore areas much wider than their final targets (Gao et al., 1999). Then, in the course of adolescence, more synapses are eliminated than formed (Zuo et al., 2005b), in part through activity-dependent processes (Hua and Smith, 2004). Synaptic pruning during adolescence is accompanied by a reorganization of neuronal connections whereby mistargeted axons and unused synapses are eliminated, and connectivity becomes more specific. The decrease of synaptic density during adolescence, which is reflected in changes in grey matter, proceeds asynchronously in different brain areas (Paus,

2005), in line with the maturation of specific cognitive functions (Shaw et al., 2006b).

Changes in synaptic density are paralleled by changes in slow wave amplitude (Huttenlocher, 1979; Feinberg, 1982; Huttenlocher and Dabholkar, 1997) and brain metabolism, presumably due to the increased energy requirements associated with increased synaptic activity (Chugani, 1998). This observation has been confirmed both in humans and in rats (Nakamura et al., 1999; Glantz et al., 2007). As suggested by the synaptic homeostasis hypothesis (Tononi and Cirelli, 2006), and confirmed by computer simulations and experimental studies in both humans and rats, changes in synaptic efficacy can account for the observed changes in sleep slow waves (Esser et al., 2007; Riedner et al., 2007; Vyazovskiy et al., 2007; Vyazovskiy et al., 2009b; Vyazovskiy et al., 2009a; Olcese et al., 2010). Thus, sleep SWA could be taken as a reliable indicator of net changes in average synaptic density/strength both in the course of the night (sleep homeostasis) and in the course of development.

Investigation of sleep SWA topography during childhood and adolescence confirmed this assumption, by showing that the location on the scalp exhibiting maximal SWA changed during development by following a posterior to anterior time course (Kurth et al., 2010a) (Figure 2). This posterior to anterior-shift is well known from MRI studies, reporting a similar time course for grey matter volume change during development (Giedd, 2004; Sowell et al., 2004; Giedd, 2008). Thus the changes in SWA topography probably reflect synaptic changes accompanying the pruning process during cortical maturation (Figure 2). Another link between cortical maturation and slow waves arises from a study that compared the SWA decrease during adolescence with alterations in grey matter volume (Buchmann et al., 2010). Both factors were positively correlated. Furthermore this relationship was most pronounced in cortical areas maturing during adolescence. An interesting aspect concerns sex differences: It has been reported that average delta power was significantly lower in girls than in boys at the age of 12-14 years, while at age 9-11 years no sex differences were observed (Feinberg

et al., 2006). This has been explained by the earlier pruning of frontal grey matter in girls (Giedd et al., 1999).

Plasticity-dependent changes in SWA

Evidence for a link between SWA and plastic changes not only arises from maturational studies but also from settings in which synaptic changes are triggered experimentally. High-density EEG recordings in adults show that reduced motor activity due to arm immobilization during the day is followed by a local decrease of SWA over the corresponding motor region compared to a normal night (Huber et al., 2006), while potentiation of synapses in the motor cortex with transcranial magnetic stimulation leads to a local increase of SWA (Huber et al., 2007b), indicating a direct relationship between synaptic strength and SWA. In another study of the same author high-density EEG recordings showed that also learning a visuomotor task, compared to a control non-learning task, produces an increase in SWA which is localized to the brain region (right parietal cortex) that is known to be involved in learning the task (Ghilardi et al., 2000; Huber et al., 2004). The subjects were trained on a rotation adaptation task where they had to reach for visual targets using a handheld cursor while unconsciously adapting to an imposed rotation. Performance, measured as the degree of deviation to the straightest movement (directional error), improved not only during the training phase before sleep but also at retest after sleep. Remarkably, when performance after sleep was related to SWA, the size of the local SWA increase during the first thirty minutes of NREM sleep predicted the decrease in directional error at retest after sleep. In another study, which also investigated the relationship between sleep SWA and task performance, participants were trained on the same learning task (Landsness et al., 2009). However during subsequent sleep they were deprived of slow waves by means of acoustic stimulation. In this case no increase of SWA over the corresponding region was observable and also no learning improvement took place (Landsness et al., 2009). From these studies it can be concluded that changes in SWA not only reflect changes in synaptic plasticity but also affect performance. Likewise the causal relationship between SWA and test

performance is not limited to the visuomotor modality but was also found in a texture discrimination task (Aeschbach et al., 2008).

Recently it was shown that the beneficial effect of sleep on visuomotor performance is independent of the time of day the task is being trained (Maatta et al., 2010). Subjects were trained in the morning instead of right before sleep and allowed to pursue their normal daily activities. Similar to previous studies, SWA was locally increased over the trained region during the subsequent night and improved performance was found during retest the following morning. However, independency of timing might be task specific as several other memory tasks only demonstrate sleep-dependent performance improvement, when sleep follows training closely (Gais et al., 2006; Talamini et al., 2008; Van Der Werf et al., 2009).

There is increasing evidence that sleep dependent performance improvement can not only be experimentally inhibited by the suppression of slow waves, but also be boosted by the stimulation of slow oscillations. Stimulation of oscillating potential fields by transcranial application of oscillating potentials (0.75 Hz) during the first NREM sleep lead to enhanced improvement in a declarative memory task compared to sham stimulation (Marshall et al., 2006). These findings further support the hypothesis that SWA plays an active role in the regulation of cortical synaptic strength.

Recently a simplified version of the learning task as was used in Huber et al. (2004) was applied in a sample of children and adolescents, ranging from 8 to 20 years. The results showed that the beneficial effects of sleep on task performance as well as the corresponding local increase in SWA is not only found in adults but already present in children and adolescents (Ringli et al., 2009).

In summary, there is good evidence that sleep SWA is a reliable indicator of net changes of synaptic strength in the course of a night (sleep homeostasis), which seems directly related to the observed post-sleep performance improvements. In the next section we will discuss how age-dependent changes in SWA may be related to behaviour and cortical maturation during development.

SLOW WAVES AND THEIR RELATION TO BEHAVIOUR

Cognitive skills

There is a large body of evidence showing that full sleep deprivation as well as part-time or chronic sleep restriction cause impairment in cognitive functioning (e.g. (Banks and Dinges, 2007). Also it is well-known that the consequences of restricted sleep duration are mainly reflected in an increase of SWA during the recovery night (Borbély and Achermann, 2005), implying a relationship between SWA and cognitive impairment.

If sleep and especially slow wave sleep does play a critical role in brain development and learning, then sleep disorders, sleep restriction, and sleep loss early in life may impair cognitive functioning. Some evidence in favour of such a relationship is becoming available. For example, a positive correlation between increased sleep/earlier bedtimes and higher school grades was found in a representative population of high school students (Wolfson and Carskadon, 1998). Moreover, actigraphy, an objective measure for evaluating sleep patterns, revealed that sleep fragmentation correlates significantly with daytime sleepiness, attentional deficits and learning impairments (Sadeh et al., 2000). Such effects seem to be more evident in younger children (Sadeh et al., 2002), possibly suggesting that sleep is even more important for neurobehavioural functioning at a younger age.

As introduced in 4.3 the predominance of SWA on the scalp parallels cortical brain maturation, originating over posterior areas during childhood and shifting forward to frontal sites during puberty (Giedd, 2004; Sowell et al., 2004; Shaw et al., 2008; Kurth et al., 2010b). Interestingly, many cognitive and behavioural functions related to the frontal cortex do not mature until late adolescence (Luna and Sweeney, 2004). Progressive maturational changes in performance of cognitive demanding tasks can be seen from childhood to adulthood. This cognitive development is thought to rely on pruning processes as well as myelination of fibre tracks (Luna and Sweeney, 2004).

In Figure 2, evidence for a link between maturation, SWA topography and behaviour is illustrated for an early and a late state of development. For example, maturation of the visual cortex occurs early in life as is shown by the decrease of synaptic density, which already starts in the first year after birth and reaches adult levels shortly before puberty (Huttenlocher and Dabholkar, 1997). Consistently SWA is highest over the occipital cortex during the same time (Kurth et al., 2010a). At the behavioural level maturation of the visual cortex is accompanied by the specification of visual skills. For example visual acuity, a function located in the occipital cortex, is developed during the first years of life and reaches adult levels at around three years (Teller, 1981). On the other hand, a set of cognitive abilities, subsumed under the term executive functions, are known to mature later in life. Executive functions are mainly controlled by the frontal cortex, a brain region maturing at a later stage of development. Synaptic density in the frontal cortex decreases around the age of four years and continues to decline until late adolescence (Huttenlocher and Dabholkar, 1997). Again theses changes on the synaptic level are reflected in the sleep SWA whose maximal values are located over central to anterior derivations at the beginning of puberty and shift more and more to the frontal cortex during the teenage years (Kurth et al., 2010a). Paralleling brain maturation, executive functions, are developed during puberty. Amongst others, executive functions can be investigated using saccadic task performance (Munoz et al., 1998). Young children exhibit high error rates, when asked to look in the opposite direction of an appearing stimulus, while during puberty, the ability to suppress reflexive saccades is progressively developed and consequently error rates decrease (Munoz et al., 1998).

However, the exact temporal relationship between brain maturation, SWA topography and behaviour still needs to be investigated.

Mental and neurological developmental disorders

Several mental and neurological disorders during development seem to relate to sleep. Thus, there is an increasing number of such disorders in which sleep was investigated. For example, in patients

with Williams syndrome (WS), a neurodevelopmental genetic disorder, characterized by distinctive cognitive impairments and physical abnormalities (Gombos et al., 2010). More than 50% of WS individuals are also diagnosed with attention-deficit hyperactivity disorder (ADHD) (Morris and Mervis, 2000; Leyfer et al., 2006). Sleep SWA was investigated in a study with participants between ages 14 to 28 years. When compared with age- and sex-matched healthy controls, WS showed increased SWA in frontal derivations. Based on the finding that absolute SWA decreases in the course of development (Feinberg et al., 2006; Campbell and Feinberg, 2009; Kurth et al., 2010b), this result might reflect delayed brain maturation in WS.

ADHD is defined by difficulties in sustaining attention and/or hyperactivity and is the most common disorder in childhood (Olfson, 1992). Its relationship to sleep is illustrated by the observation that short sleep duration and sleeping difficulties are predictors of the occurrence of ADHD symptoms (Paavonen et al., 2009). Unfortunately, quantitative investigations of the the sleep EEG (e.g. SWA) are still missing in children with ADHD. The idea of a maturational lag as the underlying cause of the disorder has been proposed by several researchers (Kinsbourne, 1973; Drechsler et al., 2005; Shaw et al., 2007; Gustafsson et al., 2010) and is supported by behavioural (Drechsler et al., 2005) and imaging studies (Shaw et al., 2006b; Shaw et al., 2007). Thus, in the light of the close relationship between cortical maturation and sleep SWA (Kurth et al., 2010a) it might be interesting to investigate the sleep EEG more closely. Specifically, it might be possible that such a developmental delay in ADHD appears in the topographical distribution of SWA, by depicting a pattern typically seen in children of younger age (Kurth et al., 2010a) or alternatively may be expressed by changed levels of absolute SWA.

Schizophrenia often emerges during or shortly after adolescence. A common phenomenon of the illness is a large reduction of amplitude of slow waves, from which it was hypothesized that some kinds of Schizophrenia may result from excessive synaptic loss in adolescence (Feinberg, 1982). Confirming this assumption it was

shown that indeed patients with childhood-onset Schizophrenia (COS) show an altered pace of neurodevelopmental trajectories with increased velocity in grey matter loss during adolescence (Gogtay and Rapoport, 2008; Rapoport and Gogtay, 2008).

Although nearly all mood disorders express co-occurring abnormalities of sleep, the relationship between sleep and emotion during development is only sparsely investigated. Sleep disturbances in adults suffering from major depression disorder (MDD) are very frequent. Findings in adult patients reported disturbed slow wave sleep in men, whereas no impairments were found in depressed women (Reynolds et al., 1990). Moreover, sleep deprivation has the potential to temporally reduce depressive symptoms in patients with major depression (Giedke and Schwarzler, 2002). Interestingly a gender difference was also found in a study with children and adolescents, showing that during puberty depressed adolescent boys exhibit much larger reduction of slow waves sleep than female patients or healthy controls (Robert et al., 2006).

Slow waves are also of interest in the context of epilepsy, since the generation of slow waves and the spike wave complexes, which are a typical feature of electrical status epilepticus during slow wave sleep (ESES), share common mechanisms. It was shown that the degree of synchrony of cortical neurons progressively increases from a pre-seizure sleep pattern to spike wave seizures (Steriade and Amzica, 1994). Onset of ESES typically occur around 4-5 years and last for several month or years, but resolve before adulthood without treatment. Usually there is a striking activation of spike waves when falling asleep, occurring during more than 85% of NREM sleep (Tassinari et al., 2005). Children suffering of continuous spikes and waves during slow wave sleep (CSWS), a kind of epileptic encephalopathy, go through a progressive deterioration of cerebral functioning (Tassinari et al., 1977). Recently, slow wave sleep of patients with ESES was studied and compared to age-matched, healthy children (Bölsterli et al., 2011). The authors analyzed the slope of the slow waves, a recently introduced marker of the degree of synchronization of the firing of cortical neurons (Riedner et al., 2007; Vyazovskiy et al., 2009b). As expected from findings in adults,

in healthy children the slope of slow waves decreased from the first to the last hour of NREM sleep, while patients showed no significant change in slope across the night. In light of the synaptic homeostasis hypothesis, this finding may indicate a disruption of the downscaling process during sleep, which may be related to the neuropsychological regressions that go along with the disorder.

DISCUSSION OF THE INVERTED U-SHAPE TIMECOURSE OF SWA

In this chapter we have highlighted the evidence for a close relationship between sleep SWA and cortical maturation. However, it is not known whether the age dependent SWA changes precede or follow the cortical changes. Longitudinal studies with MRI and sleep EEG data of the same subject may help to answer this question. Thus, uncovering the temporal relationship between sleep SWA and brain maturation may help to understand the mechanism underlying this relationship. Are slow waves merely reflecting the synaptic changes or are slow waves playing an active role in changing synapse density? If changes of SWA follow the maturational changes chronologically, this would speak for a mirroring role of SWA. However, if cortical changes follow changes of SWA during development, this may speak for an active role of SWA in cortical maturation. In Figure 3 we propose a model of how slow waves could play an active role in cortical maturation.

In adults, in the long run and under normal circumstances, there is a balance between synaptic strengthening and synaptic downscaling. Thus, during wakefulness, in an experience dependent manner synapses are strengthened and during sleep, again in an experience dependent manner, synapses are weakened.

This regulation of synaptic strength is the key mechanism of synaptic homeostasis: Attaining a sustainable level of synaptic strength, which allows learning processes to occur throughout life. There is indeed increasing evidence that synaptic strength is in balance in adult organisms (Tononi and Cirelli, 2006).

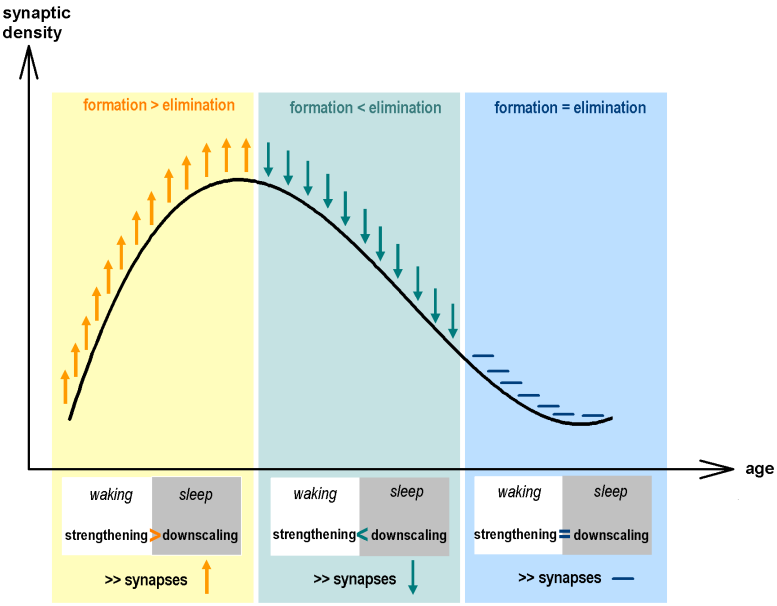

Figure 3: Illustration of the timecourse of synaptic density (y-axis) at various ages (x-axis). *Coloured areas depict roughly different periods of development: childhood/prepuberty (in yellow), adolescence/postpuberty (in green) and adulthood (in blue). In each period, mechanisms of synaptic plasticity during waking and sleep (indicated at the bottom) favour the strengthening, respectively weakening of synapses and influence the formation, respectively elimination of synapses (indicated at the top). In the first two decades of human life, dramatic changes in the number and density of synapses take place. Because more synapses are formed than eliminated, synapse density increases during childhood, reaches a maximum before puberty and decreases exponentially during adolescence, as more synapses are eliminated than formed (Zuo et al., 2005). Thus, it may be speculated that during development the balance of strengthening/formation and weakening/elimination is tilted: In the early years synaptic strengthening prevails over synaptic downscaling leading, in the long run, to a build up of synapses. On the other hand during adolescence, synaptic downscaling would outweigh synaptic strengthening and, correspondingly leading to a decrease in synapses. In adults, in the long run and under normal circumstances, there is a balance between synaptic strenghtening and synaptic downscaling (Tononi & Cirelli, 2006) and the total number of synapses remains stable (Huttenlocher & Dabholkar, 1997).*

This balance of synaptic strength (i.e. physiological plasticity) may also apply for the number of synapses (i.e. structural plasticity) given that also the formation of synapses takes place in an experience

dependent manner (Knott et al., 2002; Holtmaat et al., 2006) and there seems to be a continuum between strengthening and formation of synapses (Knott et al., 2006). Such a balance of structural plasticity fits well to the observation that in adults the total number of synapses remains stable (Huttenlocher, 1979) even though there is a significant turnover of synapses (Zuo et al., 2005b). The time window of interest is an important parameter determining whether physiological (e.g. via phosphorylation/de-phosphorylation or AMPA receptor turnover) or structural (e.g. via spine and synapse formation) changes take place. Physiological changes in synaptic strength (e.g. via LTP/LTD mechanisms) are rather fast. On the other hand, structural changes like the formation of new synapses may take longer (Trachtenberg et al., 2002). In summary, in adults, both physiological (synaptic strength) and structural (number synapses) plastic changes seem to be carefully balanced.

A different picture emerges during development. On the structural level dramatic changes in the number/density of synapses take place in the first two decades of human life (Huttenlocher, 1979; Huttenlocher and Dabholkar, 1997). Synapse density increases during childhood, reaches a maximum before puberty and decreases exponentially during adolescence. More specifically, during childhood more synapses are formed than eliminated, which is then reversed during adolescence, during which more synapses are eliminated than formed (i.e. pruned) (Zuo et al., 2005b). A numerical example illustrates how these age dependent changes in the formation and elimination of synapses may relate to sleep: during development, when 100 synaptic units are newly formed, presumably during wakefulness, only 99 are downscaled/eliminated during sleep, resulting in an overproduction of 1 unit per day. Although this difference is only small and the dysbalance may be hardly noticeable this balance shift results in a slow but steady increase of the number/density of synapses over months and years. A consequence of this steady increase in the number of synapses might be increasing network synchronization, which would result in a corresponding increase in SWA over time. During puberty the opposite may occur: 100 synaptic units are newly formed during the day but 101 are downscaled/eliminated during the night, therefore

leading to a slow but steady decrease of the number/density of synapses over time. Thus, it may be speculated that during development the balance of strengthening/formation and weakening/elimination is tilted: In the early years synaptic strengthening prevails over synaptic downscaling leading, in the long run, to a build up of synapses. On the other hand during adolescence, synaptic downscaling would outweigh synaptic strengthening and, correspondingly leading to a decrease in synapses. Which factors would be responsible for such a balance shift is unknown. Uncovering them might be an important scientific achievement, because the structural remodeling during development may be susceptible to interfering factors – whether genetic, epigenetic, environmental, or a combination thereof – leading to an increased risk for the emergence of structural, functional and ultimately behavioural abnormalities. In fact, a large body of evidence indicates that adolescence is characterized by an increasing incidence of psychiatric disorders. These include schizophrenia as well as mood, anxiety, eating, substance abuse and personality disorders (Feinberg, 1982; Keshavan et al., 1994; Saugstad, 1994; Lewis and Levitt, 2002; Paus, 2005; Blakemore, 2008).

Although direct experimental evidence for this hypothesis is lacking it opens new perspective for future research. To validate our hypothesis an animal model is needed which allows the investigation of alterations at the molecular level, the structural level at the synapse, and the surface EEG at the same time. Moreover, to establish causality direct manipulations of either the slow wave generation or synapse turnover during development are needed. Such experiments will help answering the critical question of whether sleep only reflects cortical maturation or if it actually triggers maturational processes.

CONCLUSION AND FUTURE PERSPECTIVES

The number of studies investigating sleep in childhood and adolescence is growing. This is important since sleep changes markedly from infancy to adulthood i.e. sleep duration, sleep architecture, sleep slow wave characteristics and their topography.

The understanding of sleep during childhood is important as it contributes to the understanding of the function of sleep per se.

Of special interest is the fact that during development none of the classical frequency bands changes as dramatically as the SWA band (Kurth et al., 2010a). The change of the amplitude of slow waves parallels the number of synapses (Feinberg, 1982; Huttenlocher and Dabholkar, 1997), i.e. reduced synaptic density following pruning is reflected by a decline in amplitude. And the location over which maximal SWA can be measured, undergoes a shift from posterior to anterior regions across childhood and adolescents, matching the time course of cortical maturation, as known from MRI and behavioural studies (Luna and Sweeney, 2004; Shaw et al., 2008; Kurth et al., 2010b), most likely reflecting cortical plasticity during development.

In the future examination of SWA topography in patients with neurological or mental disturbance may help to uncover the pathophysiological mechanisms underlying certain developmental disorders. However, to establish a causal relationship between cortical maturation and changes in SWA during development an animal model is needed.

The use of HD EEG measures confirmed earlier findings that SWA not only reflects global changes in synaptic density but also mirrors the regional aspects of cortical maturation (Kurth et al., 2010a). This observation is of importance because it shows the potential of HD EEG as a diagnostic tool. In clinical settings the assessment of developmental delays or cortical abnormalities is essential. However, to date the use of imaging techniques in children is limited or problematical due to the application of radioactive tracers or x-rays. Also newer techniques as MRI, which are free of radiation exposure, are expensive and difficult to apply in children, because the needed quiescence often is only reached by sedation. In contrary EEG offers several advantages as its use is cheap, free from any medical risk and offers unlimited application. Thus measurement of sleep SWA could become a powerful tool to investigate cortical maturation in health and disease.

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Research Part II:
SWA and cortical maturation

3

EEG sleep slow wave activity as a mirror of cortical maturation

Andreas Buchmann, Maya Ringli, Salomé Kurth, Margot Schaerer, Anja Geiger, Oskar G. Jenni & Reto Huber

Child Development Center, Children's University Hospital Zurich, 8032 Zurich, Switzerland

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Abstract

Deep (slow wave) sleep shows extensive maturational changes from childhood through adolescence, which is reflected in a decrease of sleep depth measured as the activity of electroencephalographic slow waves. This decrease in sleep depth is paralleled by massive synaptic remodelling during adolescence as observed in anatomical studies, which supports the notion that adolescence represents a sensitive period for cortical maturation. To assess the relationship between slow wave activity (SWA) and cortical maturation we acquired sleep EEG and MRI data in children and adolescents between 8 and 19 years. We observed a tight relationship between sleep SWA and a variety of indexes of cortical maturation derived from MR images. Specifically, grey matter volumes in regions correlating positively with the activity of slow waves largely overlapped with brain areas exhibiting an age-dependent decrease in grey matter. The positive relationship between SWA and cortical grey matter was present also for power in other frequency ranges (theta, alpha, sigma and beta) and other vigilance states (theta during REM sleep). Our findings indicate a strong relationship between sleep EEG activity and cortical maturation. We propose that in particular sleep SWA represents a good marker for structural changes in neuronal networks reflecting cortical maturation during adolescence.

Introduction

Sleep is one of the oldest and most tantalizing enigmas of neuroscience. While sleep functions are still largely unknown, its importance is highlighted by its ubiquity in the animal kingdom (Tobler, 2005; Allada and Siegel, 2008; Cirelli, 2009). When we sleep, we go through a characteristic sequence of non rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. During the deep part of NREM sleep, also called slow wave sleep, cortical neurons exhibit slow oscillations (Steriade et al., 1993). They are characterized by an alternation of depolarized up-states and hyperpolarized down-states, which can be measured as slow waves in the electroencephalogram (EEG). The activity of these slow waves (i.e. slow wave activity, SWA, EEG power between 1 and 4.5 Hz) is a well established electrophysiological measure for sleep depth (in terms of the difficulty to awaken an individual (Borbély and Achermann, 2005)). Moreover, we know that sleep slow waves are regulated homeostatically: SWA increases in proportion to the time spent awake and decreases during sleep (Borbély and Achermann, 2005). At a mechanistic level, sleep SWA reflects synaptic number and efficacy because more or stronger synapses favour neuronal synchronization (Esser et al., 2007; Vyazovskiy et al., 2009b).

Intriguingly, SWA varies strongly with age: In the first years of life, SWA increases with a maximum before puberty and then decreases during adolescence into adulthood, as reported recently using longitudinal data (Campbell and Feinberg, 2009) and earlier with cross-sectional data (Jenni and Carskadon, 2004). Interestingly, a similar time course has been demonstrated for brain energy consumption (Chugani, 1987). Furthermore, synaptic density in the prefrontal cortex exhibits the same pattern of increase during childhood and subsequent decrease across adolescence (Huttenlocher and Dabholkar, 1997). Thus, as demonstrated in many species, including man, rhesus monkey, cat, and mice, the maturation of neural circuits in the cerebral cortex is characterized by an initial overproduction of synapses, followed by a net elimination or pruning during the pre-adult years (reviewed in (Rakic et al., 1994)).

The process of elimination or stabilization seems to be steered by a complex interaction between signal molecules and neuronal activity (Innocenti and Price, 2005). Interestingly, the maturation of dendritic trees and the sizes of the pyramidal cells in the dorsolateral prefrontal cortex also show an inverted u-shaped curve, in a layer-specific pattern with a later peak in layer III than in layer V (Petanjek et al., 2008). The extent of this pruning process is exemplified by an electron microscopy study in macaque monkeys showing an estimated loss of 5000 synapses per second during the pre-adult years (Bourgeois and Rakic, 1993). This massive synaptic remodelling during adolescence supports the notion that adolescence represents a sensitive period for cortical maturation.

While synaptic density can only be studied post mortem in humans, changes in grey matter volume may be tracked in vivo with magnetic resonance imaging (MRI). Studies have reported volume increases and subsequent decreases across wide parts of the cortex, with regionally specific peak times: Lower-order somatosensory or visual cortices peak earlier than higher-order association cortices (Giedd et al., 1999; Gogtay et al., 2004; Elston et al., 2009; Tamnes et al., 2010). In summary, the age related changes in sleep SWA show the same pattern as the metabolic and synaptic changes seen during adolescence. Thus, this observation may imply that SWA is a marker of cortical maturation during the sensitive period of adolescence, as already pointed out by Campbell and Feinberg (Campbell and Feinberg, 2009).

We therefore investigated the relationship between sleep SWA and anatomical markers of cortical maturation during adolescence in the same subject population in a cross-sectional study. To do this, we collected all-night sleep EEG during 1-2 nights of sleep using a 128-channel high-density EEG system and anatomical MRI data from a group of 41 healthy children and adolescents between 8 and 19 years of age and addressed the following questions: i) Do sleep SWA and cortical grey matter volume decrease in an age-dependent manner in our subjects? ii) Are the changes in sleep SWA and grey matter volume during adolescence correlated? iii) Do correlations between

the sleep EEG and grey matter volume show regional (lobes) and frequency specific aspects?

Materials and Methods

Subjects

Of 41 subjects enrolled in the study, we excluded one subject, who was a habitual short sleeper (sleep duration shorter than the 95th percentile of the age group); two subjects left the study before we could collect sleep EEG data, resulting in 38 subjects with at least one night of EEG data. Another two subjects were excluded from the correlations with MR data, because one refused the MRI scan and the image quality of one subject was very low due to technical problems with the scanner. The final sample consisted of 36 children and adolescents (mean age: 13.5 years, SD: 3.3y; 15 girls, 21 boys). We defined puberty between age 10 and 15.9 years (based on the Tanner score, (Tanner, 1962) and adolescence between 16 and 19 years. Written informed consent was obtained from participants and parents. The study was approved by the local ethics committee according to the declaration of Helsinki.

Sleep EEG

Sleep recordings for two nights were performed by means of a 128-channel EEG amplifier (Electrical Geodesics Inc.). The two nights were separated by at least one week and took place on the same day of the week. Sleep EEG recordings in girls with menstruation were performed during the follicular phase (except for one girl). All subjects but one were recorded for the entire night. In one subject the electrodes were taken off after five hours due to discomfort. EEG recordings were sampled at 500 Hz and band-pass filtered between 0.5 and 50 Hz (except for one subject, where a 0.75 Hz high-pass filter was used to suppress low frequency sweating artefacts). Sleep stages were visually scored for 20-s epochs according to AASM criteria (Iber et al., 2007). Mean sleep latency of both nights was 20.2 minutes (SD: 12.4). Amount of sleep in stage 2 was 151.9 minutes (SD: 36), amount in sleep stage N3 was 84.7 minutes (SD: 31) for the first 4 sleep cycles.

For a quantitative analysis of the sleep EEG, a spectral analysis of consecutive 20-s epochs (FFT routine, Hanning window, averages of five 4-s epochs) was performed for 109 channels (excluding the channels below the ears) over 4 NREM sleep episodes after visual and semiautomatic artefact removal (except for one subject, who slept for 2 whole cycles only). Analyses were performed with the software package MATLAB (The Math Works, Inc., Natick, MA). With the aim to optimize stability across nights, in a first step, we varied the number of cycles (1 to 4) and stages (N2 or N3 or N2 and N3) for which we calculated SWA (EEG power between 1 and 4.5 Hz). The most stable composition was SWA for stage N2 and N3 for the first four NREM sleep episodes. In a next step we averaged the two nights. In six subjects only one night was included due to missing data, or bad data quality. Mean difference in SWA between nights was 15.1% for the electrode C4 (Pearson's $r=.977$, $p<.001$, $n=32$). SWA measures were logarithmized to obtain a linear relationship with age. Age related changes were analyzed in all 19 10-20 electrodes (Fp1, Fp2, Fz, F3, F4, F7, F8, C3, C4, T3, T4, T5, T6, P3, P4, Pz, O1, O2, Oz).

To assess the stability of the relationship between SWA and grey matter during the night, we calculated SWA for the first cycle and the fourth cycle separately. The subject with only two cycles was excluded from this analysis.

We assessed the frequency specificity of the results by separate analyses for average absolute power in the following EEG-bands (for sleep stage N2 and N3 of the same time window): low delta (0.75-1.5 Hz), high delta (2-4.5 Hz), theta (5-7 Hz), alpha (8-11 Hz), sigma (12-15 Hz), beta (20-25 Hz).

To assess the sleep state specificity of the results, we compared theta power (5-7 Hz) during NREM sleep with theta power during REM sleep for the same time intervals (first four sleep cycles, electrode C4). We used theta power for this comparison because it predominates REM sleep.

Structural MRI

All images were obtained on the same 3T scanner, a General Electrics Signa HDx. We used a T1-weighted gradient-echo whole brain image, TR 8.928ms, TE 3.496ms, flip angle 13°; image resolution in x-y-z direction was 256x256x140 voxels, resulting in a resolution of 0.938x0.938x1.2mm. The T1 images were further processed using standard methods. However, because MR-based evaluation methods have never been anatomically validated in children and adolescents, we followed a precautionary approach and used two very different methods, which both should be suitable for the evaluation of children's data: A voxel-based (VBM) analysis using a priori-maps tailored for the children's data included in our study and a surface based analysis that uses limited a priori knowledge of the brain anatomy.

VBM Analysis

Global grey and white matter volumes (as well as grey/white matter ratios, which were the simple ratio between the volume of all grey matter voxels by all white matter voxels) and whole brain grey matter maps were calculated with the SPM5 software (http://www.fil.ion.ucl.ac.uk/spm) for Matlab, using the VBM5 toolbox (http://dbm.neuro.uni-jena.de/vbm/vbm5-for-spm5) by Christian Gaser. Images were individually masked for non-brain tissues, normalised, segmented and HMRF (Hidden Markov Random Field) corrected using custom made a priori maps. Images were modulated to reverse nonlinear transformations used for the normalisation and then smoothed with a 10mm FWHM Gaussian kernel.

The custom made a priori maps were obtained by averaging segmented images of all subjects used in the study, normalized using the same procedure with a priori maps contained in SPM5 (MNI space) and smoothing the mean images for grey matter, white matter and cerebrospinal fluid with a Gaussian kernel of 10mm FWHM.

Image statistics were calculated on a voxel-by-voxel basis. Gaussian distribution of the data is provided by the smoothing of the images. Images were masked explicitly using a relative threshold of 0.1 to exclude voxels that are unlikely to belong to the grey matter segment.

Differences lying outside the brain (meninges are not fully excluded by the procedure) or between white matter and liquor were excluded.

Surface-Based Analysis

Local grey matter volumes (cortical and subcortical), cortical areas, thicknesses and curvatures were calculated using Freesurfer version stable v4.5.0 for Mac OS 10.5.2 (http://surfer.nmr.mgh.harvard.edu; see also (Dale et al., 1999; Fischl et al., 1999). In this software the T1 images are analyzed using the recon-all -all procedure, which treats subcortical structures and cortical hemispheres separately. The procedure for the cortical hemispheres uses intensity gradients to model grey-white border and measures cortical thicknesses perpendicularly to the grey-white border up to the outer liquor space. The procedure corrects the topology automatically until each hemisphere is one contiguous sheet without holes. Extracted surfaces were checked for topological faults (holes, knots). Images had only few, very small defects, which did not have to be corrected manually. With the completed models for the hemispheres, the hemispheres are registered with a spherical atlas, which utilizes individual cortical folding patterns to match cortical geometry across subjects, which allows parcellation of the cerebral cortex in to gyral and sulcal units. It has been shown that Freesurfer statistics are robust against white noise in the images, and that results are similar if using multiple T1 images to using one image (Han et al., 2006; Jovicich et al., 2009). Since the measured thicknesses have a small variability and are consistent with anatomical studies (i.e. they are slightly thicker than adult values and converge against adult values) and the measured gyral and sulcal volumes are consistent with adult values, we think that the method is valid for our age-range. Finally, Freesurfer statistics were evaluated with SPSS 16.0 for Windows.

Lobe-Wise Analysis

Areas from the surface-based analysis were fused to form the four main lobes (prefrontal, temporal, parietal and occipital lobes) left and right. The orbitofrontal cortex was not included in the prefrontal lobe.

Statistics

EEG power values and grey matter variables were compared with multiple correlations accounting for sex and total brain size (grey matter plus white matter plus liquor).

The statistical threshold assumed was p=.001 uncorrected for the SPM analysis and p=0.05 uncorrected for the surface-based analysis.

Co-localisation

The degree of co-localisation between the correlation spots over all predefined areas for two variables was defined as the scalar products of the beta weights for all predefined cortical areas (n=62), divided by the Euklidian length of the beta weight vectors (0=independent, 1=collinear, i.e. the cosine between the vectors). The values for the structural modalities (volume, area, thickness) were treated separately. The significance of these scalar products was assessed with a permutation test: The actual scalar product was compared with the distribution of theoretical scalar products, which were obtained as 100000 scalar products between the first vector and random permutations of the second vector. Consequently, a 5% significance level was defined as 5000 theoretical scalar products being larger than the measured scalar product.

Results

EEG SWA and Grey Matter Decrease with Age in Healthy Children.

As expected, averaged EEG power in the slow wave frequency range (SWA, 1-4.5 Hz, both nights) showed a marked decrease during adolescence, consistently with earlier studies (see introduction). The decrease was present in all 10-20 electrodes (with inter-electrode correlations between 0.852 and 0.992; all $p<0.001$), however variable in its extent. We found the largest decreases of SWA over central and parietal regions. Because of the high correlation between electrodes, in a first step, we refer to the classically used electrode C4. For this electrode, as for the others, the decrease of SWA was consistent with an exponential model, with a power loss of around 12% per year between 8 and 19 years of age (Fig. 1).

We first used the conventional VBM (voxel-wise analysis of whole-brain maps) analysis to detect changes in grey matter volume during adolescence. On the global level the changes in cortical organization are best reflected in a significant decrease of the grey-to-white matter ratio (mean 2.07; decreasing 0.03 per year). Moreover, consistent with published data, we found regional grey matter volume decreases with age by means of the VBM analysis (Fig. 2). Between the ages 8 and 19, the spots with the most significant volume decreases were found in the prefrontal lobe bilaterally, in the left anterior temporal lobe, the right posterior cingulate and in the left superior parietal lobe.

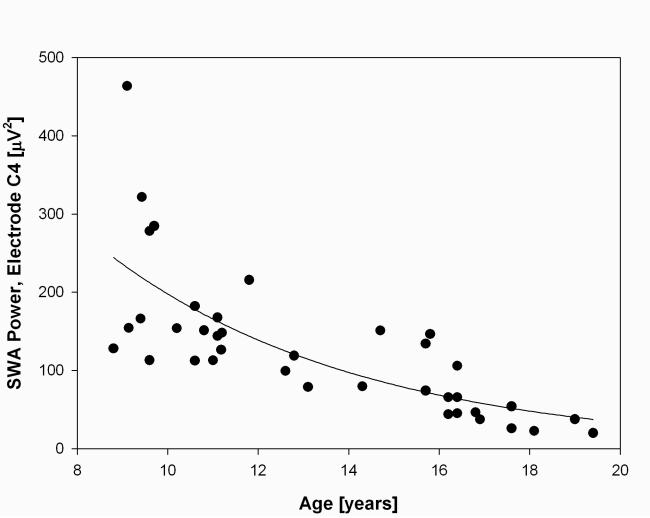


Figure 1. *Decrease of SWA (EEG power between 1 and 4.5 Hz) across adolescence (electrode C4, first 4 sleep cycles, mean over 2 nights) with a fitted exponential function.*

In the surface-based analysis, we found a small, nonsignificant global decrease of cortical thickness over the observed age range (mean 2.79 mm (SEM 0.17); decrease -0.007 mm per year, p=0.173). Analysis for the main lobes showed strongest thickness decreases with age in the parietal lobes (0.4% per year), followed by the frontal (0.3%), temporal (0.3%) and occipital (0.2%) lobes. Whereas the volume of the left orbital gyrus, the main part of the orbitofrontal

cortex, was quite stable, the right orbital gyrus showed a marked decrease in volume; this asymmetry is supported by the observation that the left-right asymmetry between the sulci increases during adolescence in this region (Sowell et al., 2002). Other individual regions with age-related increases or decreases are listed in Table 2 (last column). Most correlations with age were found for cortical thickness, where the strongest negative correlation was present for the left middle frontal gyrus (standardized beta=-0.68) and the strongest positive correlation for the right temporal pole (beta=0.54; Table 2). Thus, the observed volume changes are primarily due to changes in cortical thickness.

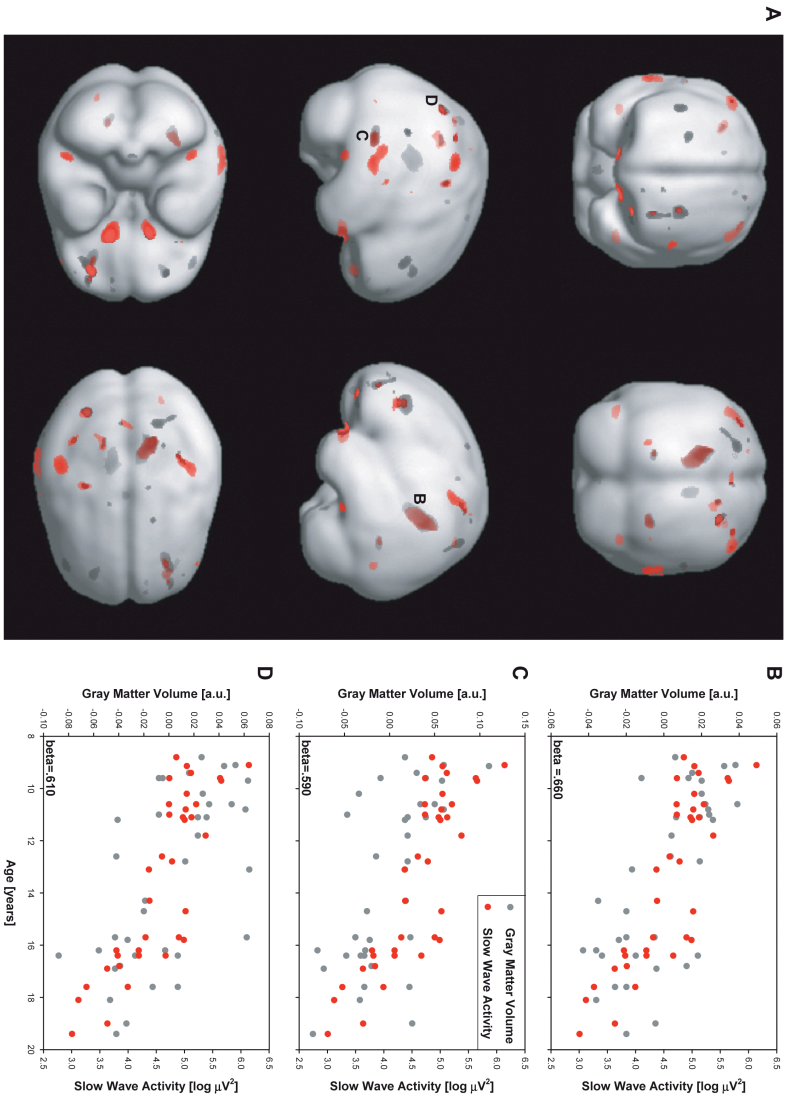
The SWA Decrease Correlates with Grey Matter Changes in Healthy Children.

Our data set allowed a direct correlation of grey matter and slow wave activity during sleep. In the whole brain analysis, the ratio between all grey matter and white matter voxels in the entire brain, which showed a marked decrease over the age range, correlated significantly with SWA at electrode C4 (beta=0.367, p=0.025).

Using VBM analysis, we found significant positive correlations between SWA at electrode C4 and grey matter volumes in the posterior cingulate, bilateral parietal and left dorsolateral prefrontal lobe as well as sensorimotor, occipital and posterior temporal areas. There were no significant correlations with the volumes of the large subcortical core structures (basal ganglia, thalamus, amygdala). Moreover, the positive correlations co-localized to a large extent with a subset of the regions showing the most pronounced age dependent decrease in grey matter (Fig. 2a; Fig. 3; see also below). The strongest positive correlations between SWA and grey matter, which overlapped with regions exhibiting age-dependent decreases in grey matter volume of the parietal (posterior cingulate), temporal (fusiform gyrus) and occipital lobes (cuneus/ precuneus border) explain up to 45% of the variability (Fig. 2b-d). Only few regions showed negative correlations between grey matter volume and SWA, i.e. the right temporal pole and the left hippocampus. Note that the hippocampus is among the few brain structures that increases in volume at this age range and might be an interesting target for further investigation

because of its role in learning and memory processes (Moscovitch et al., 2006).

As found in the VBM analysis, the surface-based analysis revealed predominately positive correlations between local grey matter measures and sleep SWA at electrode C4. We observed similar results for other electrodes with a large overlap between cortical regions correlating with SWA (data not shown, see Methods for electrode details). The main associations were found for cortical thickness and SWA, whereas surface area revealed only few associations with SWA (Table 1 for pooled N2 and N3; the correlations with separate SWA for sleep stages N2 and N3 can be found in Supplementary Table S1). Virtually all areas showing a positive correlation between grey matter volume and sleep SWA using the VBM analysis also showed a positive correlation in the surface-based analysis, i.e. for cortical thickness, but with more prefrontal regions involved. We believe that surface-based assessment of cortical thickness is the more sensitive measure for changes in cortical structure, because it does not rely on spatial correspondence of individual structures via normalization of the brain to an averaged template, but maps the individual gyri and sulci of each subject (see Methods for more details). This might be of particular importance for the complicated gyrification in the prefrontal cortex.



<Figure 2. *Correlation between cortical gray matter and sleep SWA during adolescence (mean of 2 nights, first 4 sleep cycles, electrode C4). (A) Overlay of gray matter decrease*

with age (gray) and regions with positive correlation between gray matter volume and sleep SWA (red). (B--D) Time course of gray matter and logarithmized sleep SWA for 3 example regions: (B) posterior cingulate, (C) fusiform gyrus, and (D) cuneus/precuneus border. Beta values refer to standardized weights of the multiple correlation between SWA at electrode C4 and gray matter volume, corrected for sex and whole-brain volume; P\0.001, uncorrected for multiple comparisons.

Next we tested whether the close relationship between grey matter measures and sleep SWA exists independent of age. Thus, we applied an age-correction to the multiple correlations. Even when controlling for age we found significant correlations between grey matter measures and SWA (with smaller effect sizes), again with a preponderance of positive correlations (Table 1). Another contributing factor might be head size, because it consistently showed positive correlations with SWA. Thus all calculations were corrected for whole brain volume (sum of grey matter, white matter, and liquor). Finally, the distance between the electrode and the cortical tissue might influence the signal strength. In this respect it is noteworthy that the thickness of non-brain tissues failed to show any significant effects.

To assess the relationship between the time course of SWA and cortical grey matter measures, we calculated multiple correlations between SWA in the first and the fourth sleep cycle (see methods) and parcellated grey matter measures from the surface-based analysis. For both the first and the fourth sleep cycle we obtained qualitatively similar results in terms of regions and beta weights as for the average across the first four cycles with no statistical differences (data not shown).

Table 1: *Brain regions showing significant correlations between gray matter measures and SWA at electrode C4 (beta: standardized weights of the multiple correlation corrected for sex and brain size).*

Region	Hemisphere	Measure	Beta	P (uncorrected)
Without age correction				
Inferopr frontal gyrus, triangular part	L	Thickness	0.388	0.023
	R	Thickness	0.423	0.040
Middle frontal gyrus	L	Thickness	0.572	0.029
Superior frontal sulcus	L	Area	-0.485	0.010
Sup. frontal gyrus	L	Thickness	0.476	0.026
	R	Thickness	0.623	0.014
Orbital gyrus	R	Volume	0.470	0.006
		Area	0.451	0.019
		Thickness	0.519	0.008
Middle temporal gyrus	R	Thickness	0.445	0.015
Superior temporal sulcus	R	Thickness	0.383	0.034
Fusiform gyrus	R	Thickness	0.582	0.001
Postcentral gyrus	R	Volume	0.392	0.029
Supramarginal gyrus	L	Thickness	0.409	0.014
	R	Volume	0.407	0.027
		Thickness	0.345	0.036
Intraparietal sulcus	L	Thickness	0.533	0.002
	R	Thickness	0.537	0.003
Superior parietal gyrus	L	Thickness	0.437	0.019
	R	Volume		
		Thickness	0.354	0.041
Precuneus	L	Thickness	0.506	0.006
	R	Thickness	0.475	0.007
With age correction				
Inferior frontal gyrus, orbital part	R	Volume	-0.195	0.042
	L	Area	-0.194	0.049
Orbital gyrus	L	Area	0.360	0.031
Parahippocampal gyrus	L	Volume	0.241	0.023
		Thickness	0.261	0.010
	R	Volume	0.232	0.020
Fusiform gyrus	L	Thickness	0.224	0.033
Inferior temporal sulcus	R	Volume	0.216	0.016
		Area	0.242	0.017
Central sulcus	R	Volume	0.240	0.028
Supramarginal gyrus	L	Thickness	0.271	0.012
Intraparietal sulcus	R	Thickness	0.260	0.012

Regional aspects of the correlation between MRI markers of cortical maturation and sleep SWA.

Imaging studies show a specific progression of cortical brain development reflected in a region specific time course of macroscopic grey matter changes (see introduction). Thus, in a next step, we explored the regional aspects of the relationship between grey matter measures and sleep SWA. Even though the age-related decrease in SWA was present in all electrodes we found some region-specific differences in its expression. We found the fastest decrease rate of SWA in centro-parietal (slope of the linear regression b=-32 μV2/y), occipital and dorsal frontal (b=-28) electrodes, followed by temporal (b=-23) and central and lateral frontal electrodes (b=-18 to -19). To account for the global decrease of SWA with increasing age, which might be related to the global decrease of grey matter volume, we normalized SWA at each electrode to the mean over all 10-20 electrodes to investigate regional aspects. Interestingly, we found the strongest relative decrease of SWA in parietal and parieto-central electrodes (slope of the linear regression b=-0.025), close to the area where we also found the strongest decrease of grey matter.

Moreover, we observed relative increases of SWA in frontopolar and frontocentral regions (b=0.04), which encompasses the observed grey matter volume increases in the superior frontal sulcus. Relative power in the theta and sigma frequency range did not show such a close relationship to local grey matter volume changes. With the limited spatial resolution of EEG recordings in mind, our results reveal some regional differences in the relationship between SWA and grey matter, which correspond to previously reported facets of the progression of brain development.

Region	Hemisphere	Measure	Low delta	High delta	Theta	Alpha	Sigma	Beta	Age
Inferior frontal gyrus, opercular	L	Volume					0.338		
	R	Thickness				0.354	0.351		
Inferior frontal gyrus, orbital	R	Volume							0.341
Inferior frontal gyrus triangular	L	Thickness	0.376	0.395	0.383				
	R	Thickness	0.420	0.428					0.428
Middle frontal sulcus	L	Volume		0.403	0.491	0.577	0.508	0.467	
		Area		0.374	0.447	0.514	0.477	0.427	
		Thickness		0.334		0.309			
	R	Thickness							0.369
Middle frontal gyrus	L	Thickness	0.638	0.521					0.680
Superior frontal sulcus	L	Volume							0.390
		Area	0.487	0.458	0.496	0.384			0.539
Superior frontal gyrus	L	Thickness	0.519	0.454					0.555
	R	Thickness	0.655	0.615					0.575
Orbital gyrus	R	Volume	0.482	0.453	0.388	0.370		0.359	0.532
		Area	0.455	0.441	0.377	0.436			
		Thickness	0.559	0.467	0.457				0.586
Temporal pole	L	Volume							0.465
		Thickness						0.427	
	R	Volume			0.330	0.363	0.380	0.350	0.456
		Thickness				0.563	0.463	0.524	0.539
Inferior temporal gyrus	L	Area		0.373			0.394		
Inferior temporal sulcus	L	Area		0.431	0.392				0.453
Middle temporal gyrus	R	Thickness	0.523						
Superior temporal sulcus	L	Thickness	0.360						
	R	Thickness	0.382	0.371					0.387
Cingulate gyrus	L	Thickness							
Postcentral gyrus	R	Volume	0.393	0.370	0.351				0.415
Angular gyrus	L	Thickness							
Supramarginal gyrus	L	Thickness	0.483						
	R	Volume		0.473	0.435	0.389			0.397
		Thickness	0.352	0.358					
Intraparietal sulcus	L	Thickness	0.496	0.574	0.454	0.346		0.418	0.549
	R	Thickness	0.517	0.539	0.389				
Superior parietal gyrus	L	Thickness	0.475	0.398					0.568
	R	Volume					0.370		0.490
		Area				0.424			0.406
		Thickness	0.368						0.375
Precuneus	L	Volume							0.454
		Area							0.431
		Thickness	0.561	0.472	0.433	0.429		0.443	0.523
	R	Thickness	0.530	0.435	0.472				0.400
Fusiform gyrus	R	Thickness	0.544	0.618	0.528	0.446		0.534	0.463
Parahippocampal gyrus	R	Volume							0.385
		Area							0.494
Lingual gyrus	L	Thickness					0.362		
	R	Thickness							0.402
Inferior occipital gyrus	R	Thickness			0.415	0.362			

Table 2 *Brain regions showing significant correlations between gray matter measures and power in different frequency bands for electrode C4 (beta: standardized weights of the multiple correlation corrected for sex and brain size)*

Correlation between MRI markers of cortical maturation and other frequencies of the sleep EEG.

We next examined whether the relationship between sleep EEG activity and grey matter was specific to certain frequencies. This is of interest because the most pronounced age related changes in the sleep EEG were found for the lower part of the frequency spectrum (<8 Hz; (Jenni and Carskadon, 2004)), in which activity is closely related to sleep depth. Thus, to test for the frequency specificity of our results, we investigated also other classical frequency ranges of the sleep EEG. As found in previous studies, other frequency ranges showed also age-dependent decreases in power. In our subjects we observed the steepest slopes of annual decrease in the low delta and theta range, followed by the high delta, alpha, sigma and beta range. The highest negative correlations between power and age were found in the high delta ($r=-0.728$) and low delta (-0.696) range, followed by the theta (-0.668), alpha (-0.606) beta (-0.588) and sigma (-0.538) range (all $p<0.001$). However, it is noteworthy that power values in all frequency bands depend on each other (i.e. correlations between band power ranged from $r=0.415$, $p<0.01$ for low delta and sigma to $r=0.926$, $p<0.001$ for low and high delta; all correlations between the frequency bands are provided in Supplementary Table S2). Next we correlated power in the different frequency ranges with changes in MRI markers of cortical maturation. On the global level, the grey-to-white ratios correlated only with power in the low delta (standardized beta=0.484, $p=0.003$) and high delta (beta=0.427, $p=0.009$) bands, but not with power in the higher frequency bands. On the local level, however, we found a similar preponderance of positive correlations between EEG power and grey matter volumes for all other frequency ranges, as for SWA (Table 2). To assess the significance of the overlap of age related grey matter changes and EEG power - grey matter correlations we calculated the degree of co-localisation (see Materials and Methods). Co-localisation with age-related thickness decreases was most pronounced for positive correlations with power in the slow frequency ranges (low delta and high delta) and least pronounced for the spindle frequency range (sigma), with intermediate values for alpha and beta (Fig. 3). This pattern was similar for grey matter volumes and cortical surface

areas. Co-localisations for all frequency ranges were highly significant using a permutation test ($p<0.001$; see Materials and Methods).

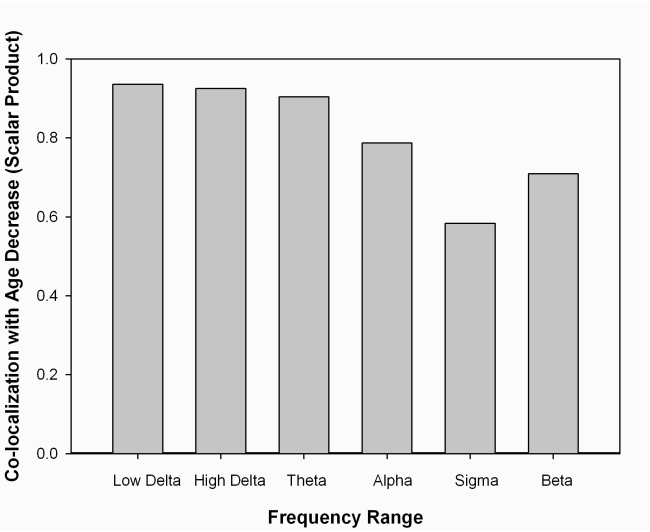


Figure 3. *Colocalization of the correlation between age and cortical thickness and the correlation between EEG power and cortical thickness for different frequency bands (scalar products; 0 means completely independent, 1 means collinear, see Materials and methods).*

Finally, we assessed sleep state specificity of our results by correlating theta power during REM sleep in the first four cycles of the night with parcellated grey matter volumes, areas and cortical thicknesses. We compared these results with the results obtained for the analogous correlations with theta power during NREM sleep during the same time period (Table 2). The results involved the same brain structures and the beta values were similar, without any significant differences (all $p>0.1$; data not shown).

Discussion

Our study shows that i) sleep SWA and cortical grey matter decrease during adolescence as observed previously ii) the decreases in SWA and grey matter are highly correlated, and iii) the relationship

between the sleep EEG and grey matter is most pronounced in areas maturing during adolescence and strongest for the SWA frequency range.

As observed in longitudinal (Campbell and Feinberg, 2009) and cross-sectional (Jenni and Carskadon, 2004) studies, we confirm in our sample that SWA decreases during adolescence. The decrease is not confined to the SWA frequency range but, though to a lesser extent, also found for all other frequency ranges. This observation is also illustrated by the inter-correlations between power of frequency bands across the entire spectrum. Also Campbell and Feinberg reported age dependent changes in the sleep EEG for other frequency ranges, i.e. for theta power (Campbell and Feinberg, 2009). Moreover, confirming other reports (Gogtay et al., 2004; Sowell et al., 2004), we observed age related changes in cortical grey matter. The changes were most pronounced in the medial parietal lobe and in parts of the prefrontal cortex – areas known to show maturational changes during adolescence.

Our data set allowed to directly evaluating the relationship between the changes in sleep SWA and cortical grey matter. As postulated by Campbell and Feinberg (Campbell and Feinberg, 2009), we observed a tight relationship between sleep SWA and a variety of indexes of cortical maturation derived from MR images. Most significant were correlations with cortical thickness in the right orbital gyrus, the right fusiform gyrus, both intraparietal sulci and bilateral precuneus. However to a lesser extent, some cortical grey matter volumes and surface areas also showed a significant relationship with SWA. It is of particular interest that the highest correlations between sleep SWA and grey matter volume/thickness were found in the same areas showing the largest changes in grey matter, i.e. the areas displaying maturational changes during adolescence. Some of these areas exhibited a significant correlation even after correcting for age, revealing that sleep SWA, in these areas, explains more variability in cortical maturation than age. This might be due to large developmental differences in our age range, and thereby signify the importance of good markers of cortical maturation. Moreover, an extension of our analysis to the first and the fourth sleep cycle

revealed a stable relationship between cortical grey matter changes and sleep SWA, which supports the validity of SWA as a marker of cortical maturation. Because whole brain volume and skull thickness might change with age and may affect the EEG signal we also looked for the influence of these variables on our results. While skull thickness failed to show a significant relationship with SWA, whole brain volume was correlated positively with slow wave activity. Thus, all reported results are corrected for whole brain volume.

The major finding of our study is the close relation of slow wave activity, which is an electrophysiological measure of sleep depth, and local cortical grey matter volumes. The coincidence between age-related cortical pruning with spots of high positive correlation between sleep SWA and local grey matter volumes suggests that the activity of slow waves during deep sleep can be used as a marker of cortical maturation. However, how may such a close relationship between age-related grey matter changes and changes in sleep SWA arise? It can only be speculated that the larger grey matter volume in children reflects, at least in part, higher synaptic density and possibly also higher cell density. There exists good evidence, however, that children have not only increased synaptic density in higher-order (association) areas, but probably also more cell activity (Brewer et al., 2009). The increased cell activity fits well to the higher brain energy consumption observed in children (Chugani, 1987). Thus, in children a specific task would involve more neurons/ synapses and more action potentials than in adults. To recruit these larger networks needed to carry out a task, input neurons should be able to influence the activity of more postsynaptic neurons. This observation might also be reflected in some common forms of epilepsy starting during childhood, which often vanish in adulthood (Neubauer et al., 2008). Thus, as a result of the spontaneous activity of a given neuron during sleep, the probability of an action potential in postsynaptic neurons is higher, leading to increased synchronicity across the network (always as compared to an adult pruned network). Higher synchronicity leads also to higher EEG power (Vyazovskiy et al., 2009). Such a mechanism may also relate to an observation when manipulating sleep homeostasis: Though the homeostatic regulation of sleep is mainly reflected in the SWA frequency range (Borbély and

Achermann, 2005) it extends also to higher frequencies. For example, the increased sleep depth after sleep deprivation is reflected in an increase of EEG power up to 10 Hz (Finelli et al., 2000). Not surprisingly, however, the strongest correlations were found for EEG power in the slow-wave frequency range given that this rhythm dominates the EEG during deep sleep. It is noteworthy that the correlation between EEG power and grey matter was especially evident within the low delta band, at frequencies corresponding to the slow oscillations (Steriade et al., 1993). Note that this mechanism is not confined to slow waves, but applies to rhythmic oscillations in general, like theta, alpha and beta waves, which should share the same anatomical substrate for the generation of cortical activity. Indeed, we found similar positive correlations with local grey matter volumes for the higher frequency ranges. Moreover, given the proposed mechanism, a relationship between electrical activity and grey matter may not be restricted to deep sleep, but apply to different behavioural states and EEG frequencies as well. Indeed, we found a preponderance of positive correlations between grey matter volumes and EEG power for N2 and N3 separately and for EEG power in the theta range also during REM sleep. This fits to the observation that theta power during NREM and REM sleep were highly correlated ($r=0.913$, $p<0.001$). In addition a similar relationship was reported for the waking EEG: Absolute power in quiet waking EEG in slow waves, alpha and beta correlates positively with grey matter in the four main lobes, most strongly at lower frequencies (Whitford et al., 2007). However, power values measured in the waking EEG are more difficult to interpret and presumably more variable, because they are strongly affected by general cognitive performance and the specificity of the task or the applied setting.

There are several limiting factors to our study. First, of course our study, which is correlative in nature, is not able to proof causalities or to exclude mediating factors between cortical grey matter and SWA. A possible class of mediators could be hormones, for example the growth hormone- insulin-like growth factor 1 (GH-IGF 1) - axis: These hormones do not only promote brain growth and influence the densities of dendritic trees (Aberg et al., 2006), but also promote sleep in rabbits and rats (Obal et al., 1988). Other possible mediators

are neurotrophins like brain-derived neurotrophic factor (BDNF), which is related to synaptic plasticity in an activity-dependent manner (Savitz et al., 2006). BDNF was increased after sleep deprivation (Hairston et al., 2004) and it is more expressed in rats showing more SWA after exploring enriched environments (Huber et al., 2007a). In addition, if injected directly into the brain, BDNF promotes sleep SWA (Faraguna et al., 2008). Second, our study is purely cross-sectional and cannot show a development of brain structure and electrical cortical activity, which could be achieved with longitudinal data. Third, changes in MRI grey matter volumes can only approximate changes in synaptic density. In humans synapse numbers can only be counted post-mortem. Hence, the valuable studies by Huttenlocher and colleagues show a clear decrease of synapse density during adolescence (Huttenlocher and Dabholkar, 1997). Using MRI to approximate changes in synapse density it seems that cortical thickness is the most sensitive measure, because, other than conventional voxel-based morphometry (Hutton et al., 2009), it tracks the changes in the thickness of several cortical layers, which have been observed in a post mortem study (Rabinowicz et al., 2009). However, MRI can assess only macroscopic anatomical structure at a scale of millimeters, but not the remodelling of synaptic structures itself, which has to be assessed with animal or post mortem human studies.

With all these limitations in mind, our study provides convincing evidence that SWA during sleep represents a good electrophysiological marker of cortical maturation during adolescence. It allows for: i) longitudinal data acquired at multiple times on the same subject, with little risk, low costs and without requiring immobility – all of these points are particularly relevant for young subjects and/or patients; ii) SWA might reflect not only synaptic density, i.e. number, but also other synaptic properties like synaptic strength and efficacy. This is elegantly illustrated by studies in rats where synaptic strength was for example measured as the level of AMPA receptors per synapse and synaptic efficacy as the magnitude of the physiological effects, e.g. postsynaptic currents (Vyazovskiy et al., 2008). Recently Vyazovskiy et al showed that both

synaptic strength and synaptic efficacy are determinants of sleep SWA (Vyazovskiy et al., 2009).

Finally, the activity of sleep slow waves may not only be a consequence of cortical maturation but also play an active role in restructuring networks. Evidence comes from the observation that rhythmic activity can induce plastic processes like synaptic remodelling both during development and learning (Katz and Shatz, 1996). Moreover, sleep may represent an ideal state to perform such remodelling (Tononi and Cirelli, 2006). Indeed studies in adults have suggested a role of slow waves in the plasticity of local circuits (Huber et al., 2004; Huber et al., 2006). It was shown that both sleep deprivation (Guzman-Marin et al., 2005) and sleep fragmentation (Guzman-Marin et al., 2007) can reduce neurogenesis in the dentate gyrus of the hippocampus. Studies in kittens have shown that the shift in ocular dominance after monocular deprivation in cats can be strengthened by sleep and correlates with the amount of NREM sleep (Frank et al., 2001). Moreover, blocking neuronal activity during sleep using Na-channel blockers resulted in a reduction of ocular dominance plasticity (Jha et al., 2005). Effects downstream of NMDA-receptor activation (probably via increased phosphorylation of the proteins CaMKII and ERK and the GluR1 subunit of the AMPA-receptor) are responsible for these plasticity effects, because blocking the NMDA-receptor abolishes the shift in ocular dominance (Aton et al., 2009). It can be assumed that the relatively large and dense cortical networks in children allow synaptic pruning, a use dependent process that makes the networks more efficient in routine processes (Hua and Smith, 2004), but less flexible for learning novel processes. As the brain becomes more efficient, the drive for restructuring might decrease, which would explain the exponentially falling curve of slow wave activity, i.e. the reduction of sleep depth, into adulthood.

No matter if sleep SWA merely mirrors cortical changes or actively interacts with this process, our study highlights the strength of sleep SWA for the exploration of maturational changes during adolescence. This is of importance because, i) adolescence is an especially sensitive period for synaptic pruning in cortical circuits involved in

cognitive functions, and ii) adolescence is also a sensitive period for the pathophysiology of many psychiatric disorders, presumably due to this extensive synaptic remodelling (Paus et al., 2008). For example, “overpruning” during adolescence, when schizophrenia symptoms often start (Keshavan et al., 1994), could explain the reduced expression of synaptic proteins and the decreased volume of neuropil in prefrontal circuits observed in schizophrenic patients (Woo and Crowell, 2005). Alternatively, pruning during adolescence may unmask pre-existing synaptic deficits (Hoffman and McGlashan 1993). Defects in pruning have also been linked to mood disorders (Saugstad, 1994), autism, and mental retardation (Tessier and Broadie, 2009). Thus, being able to monitor the extent of synaptic remodeling during adolescence could increase our understanding of pathophysiology, help early diagnosis and guide potential therapies (Woo and Crowell, 2005).

Supplemental Material

Table S1. *Gray matter correlates of SWA at electrode C4 for pooled sleep stages N2 and N3, and for N2 and N3 separately (standardized beta values for multiple regressions corrected for sex and brain size).*

region	hemisph.	measure	N2&N3 beta	N2&N3 p (uncorr.)	N2 beta	N2 p (uncorr.)	N3 beta	N3 p (uncorr.)
inf. frontal gyrus, triangular part	L	thickness	0.388	0.023			0.352	0.039
	R	thickness	0.423	0.040			0.376	[0.068]
middle frontal sulcus	L	volume	0.346	[0.070]	0.402	0.040	0.405	0.030
		thickness	0.307	[0.067]			0.322	0.049
middle frontal gyrus	L	thickness	0.572	0.029	0.520	[0.053]	0.560	0.031
sup. frontal sulcus	L	area	-0.485	0.010	-0.458	0.018	-0.460	0.015
sup. frontal gyrus	L	thickness	0.476	0.026	0.447	0.042	0.456	0.032
	R	thickness	0.623	0.014	0.443	[0.098]	0.634	0.011
orbital gyrus	R	volume	0.470	0.006	0.444	0.012	0.455	0.007
		area	0.451	0.019	0.467	0.022	0.477	0.013
		thickness	0.519	0.008	0.459	0.024	0.484	0.011
thalamus	R	volume			0.399	0.045		
middle temporal gyrus	R	thickness	0.445	0.015	0.422	0.035	0.405	0.322
sup. temporal sulcus	R	thickness	0.383	0.034			0.329	[0.069]
temporal pole	R	volume	-0.283	[0.093]	-0.404	0.018	-0.288	[0.083]
		thickness			-0.464	0.048		
fusiform gyrus	R	thickness	0.582	0.001	0.553	0.005	0.551	0.002
supramarginal gyrus	L	thickness	0.409	0.014	0.376	0.027	0.411	0.012
	R	volume	0.407	0.027	0.460	0.015	0.425	0.019
		thickness	0.345	0.036	0.281	[0.085]	0.319	0.050
intraparietal sulcus	L	thickness	0.533	0.002	0.382	0.039	0.467	0.008
	R	thickness	0.537	0.003	0.409	0.029	0.552	0.002
superior parietal gyrus	L	thickness	0.437	0.019	0.324	[0.085]	0.428	0.020
	R	volume			0.375	0.046		
		thickness	0.354	0.041	0.329	[0.053]	0.355	0.039
postcentral gyrus	R	volume	0.392	0.029	0.355	[0.057]	0.372	0.036
precuneus	L	thickness	0.506	0.006	0.559	0.002	0.480	0.009
	R	thickness	0.475	0.007	0.488	0.006	0.428	0.014

[] tendency, $0.05 < p < 0.10$

Table S2. *Pearson correlations of logarithmized mean power between frequency bands.*

	high delta	theta	alpha	sigma	beta
low delta	0.926	0.840	0.576	0.415§	0.573
high delta		0.918	0.689	0.464	0.677
theta			0.764	0.577	0.693
alpha				0.603	0.795
sigma					0.736

(all significant, p<.001; § p<.01)

4

Mapping of cortical activity in the first two decades of life: a high-density sleep EEG study

Salomé Kurth[1], Maya Ringli[1], Anja Geiger[1], Monique LeBourgeois[3,4], Oskar G. Jenni[1,2] & Reto Huber[1,2]

1) Child Development Center, Children's University Hospital Zurich, 8032 Zurich, Switzerland

2) Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland

3) University of Colorado at Boulder, Department of Integrative Physiology, Boulder, CO, USA

4) The Warren Alpert Medical School of Brown University, Department of Psychiatry and Human Behaviour, Providence, RI, USA

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Abstract

Evidence that EEG slow-wave activity (SWA, EEG spectral power in the 1-4.5Hz band) during Non Rapid Eye Movement sleep reflects plastic changes is increasing (Tononi and Cirelli, 2006). Regional assessment of grey matter development from neuroimaging studies reveals a postero-anterior trajectory of cortical maturation in the first three decades of life (e.g. (Shaw et al., 2008)). Our aim was to test whether this regional cortical maturation is reflected in regional changes of sleep SWA. We evaluated all-night high-density EEG (128 channels) in 55 healthy human subjects (2.4-19.4y) and assessed age-related changes in NREM sleep topography.

As in adults, we observed frequency specific topographical distributions of sleep EEG power in all subjects. However, from early childhood to late adolescence the location on the scalp showing maximal SWA underwent a shift from posterior to anterior regions. This shift along the postero-anterior axis was only present in the SWA frequency range and remained stable across the night.

Changes in the topography of SWA during sleep parallel neuroimaging study findings indicating cortical maturation starts early in posterior areas and spreads rostrally over the frontal cortex. Thus, SWA might reflect the underlying processes of cortical maturation. In the future, sleep SWA assessments may be used as a clinical tool to detect aberrations in cortical maturation.

Introduction

Evidence about the generation and functions of cortical activity during sleep is accumulating (Steriade, 2006; Tononi and Cirelli, 2006; Diekelmann and Born, 2010). In particular, the slow fluctuations of cortical activity during deep sleep have become a primary focus of many studies. First described by Steriade and colleagues (Steriade et al., 1993), these so-called “slow oscillations” are found in virtually all cortical neurons (Steriade et al., 2001; Timofeev et al., 2001). When slow oscillations are synchronized and involve the majority of cortical neurons in a certain region, they become visible in the surface EEG as slow waves (Vyazovskiy et al., 2009). Lately, understanding has been increasing of where slow waves originate and how they are generated during sleep (Massimini et al., 2004; Vyazovskiy et al., 2009). The activity of sleep slow waves (slow wave activity, SWA, frequency range 1-4.5 Hz) reflects the depth of sleep (Borbély and Achermann, 2005) and seem also be related to processes of brain (Sejnowski and Destexhe, 2000; Steriade and Timofeev, 2003; Born et al., 2006; Tononi and Cirelli, 2006). Interestingly, sleep slow waves exhibit substantial changes from early childhood through adolescence (Jenni and Carskadon, 2004; Campbell and Feinberg, 2009) a developmental time window with massive changes in brain morphology and function (Johnson, 2001). The activity of slow waves during sleep increases in the first years of life, reaches its maximum shortly before puberty and declines throughout adolescence (Feinberg, 1982; Gaudreau et al., 2001; Jenni and Carskadon, 2004; Campbell and Feinberg, 2009). This inverted U-shape change of SWA as a function of subject age shows similarities with the time course of synapse density, which peaks between 4 and 6 years in prefrontal cortex (Huttenlocher and Dabholkar, 1997) and with cortical grey matter volume, as tracked by magnetic resonance imaging (MRI), presumably indirectly reflecting changes in synapse density (Giedd, 2004). Such longitudinal MRI studies indicate that not all cortical regions undergo maturational changes at the same speed and time (Giedd, 2004; Sowell et al., 2004; Shaw et al., 2008). Maturation follows a postero-anterior shift across the cortex (Giedd, 2004; Sowell et al., 2004; Shaw et al., 2008). According to these

studies, occipital regions develop first and frontal areas mature last, which is consistent with the observation that many cognitive and behavioural functions related to the frontal cortex do not mature until late adolescence (Luna and Sweeney, 2004). Based on these observations, we examined whether the expression of SWA during sleep follows a similar spatial evolution during development.

Research supporting regional differences in the expression of sleep SWA stems from adults (Werth et al., 1996a; Cajochen et al., 1999; Finelli et al., 2001b; Huber et al., 2004). These studies show that SWA is most pronounced over frontal cortices (Cajochen et al., 1999; Finelli et al., 2001b)). Furthermore, the origin of slow oscillations is particularly frequent over anterior regions (Massimini et al., 2004). Regional aspects of SWA are attributed to use dependent changes (Kattler et al., 1994) or more specifically, to plastic changes induced by learning processes (Huber et al., 2004). Thus, the frontal predominance of SWA is in accordance with the observation that the frontal cortex is the brain area most “used” or plastic in adults (Horne, 1993; Couyoumdjian et al., 2010). Finally, the close relationship between cortical plasticity and sleep SWA may also be reflected by the association of slow waves and synaptic density or strength (Tononi and Cirelli, 2006). For example, using multi-unit activity recordings in rats, Vyazovskiy et al. (Vyazovskiy et al., 2009)demonstrated a relationship between sleep homeostasis and the synchronization of neuronal population activity. Specifically, in early sleep, when sleep SWA is high, they found that most individual neurons stop or resume firing in near synchrony; however, in late sleep, when SWA has dissipated, the entry into ‘on’ and ‘off’ periods was much more variable across neurons. On the other hand, computer simulations, electrophysiological and molecular data support a relationship between sleep homeostasis and synaptic strength, i.e. synaptic strength generally decreases during sleep and increases during wakefulness (Esser et al., 2007; Vyazovskiy et al., 2008). Together it seems that synchronization depends on the level of synaptic strength: The denser and stronger synapses are, the faster they synchronize their activity and the larger is the resulting potential change measured by standard EEG over the cortex.

Thus, in order to explore the relationship between the extensive remodeling of brain circuits during cortical maturation and sleep EEG activity, we collected all night high-density (HD) EEG recordings from 55 children and adolescents (age 2.4-19.4 years). We found that the location on the scalp showing maximal SWA underwent a shift from posterior to anterior regions from early childhood to late adolescence. Because anatomical maturation starts in posterior areas and spreads rostrally over the frontal cortex, we conclude SWA might reflect the underlying processes of cortical maturation.

Methods

Participants

A total of 55 healthy subjects (range 2.4-19.4y, 29 males) were recruited. Subjects underwent a telephone and questionnaire screening to exclude personal or family history of psychopathology, chronic diseases, sleep disorders, current use of psychoactive agents or other medications. No participants travelled across more than one time zone in the 4 months prior to the study. Written informed consent was obtained from the parents or from the participants of full age after explanation of the study methods and aims. The procedures were approved by the local ethics committee, and the study was performed according to the declaration of Helsinki.

Participants or parents completed daily sleep diaries. Participants also wore wrist actigraphs to ensure schedule compliance prior to the recording night. Subjects were required to refrain from alcohol and medication. No naps were allowed 24h preceding the testing, but in children used to regular napping (i.e. nap opportunity each day and falling asleep at least 4 days per week) the subjects were permitted to nap on the day of assessment so as not to introduce heightened sleep pressure (this was the case in two children of ages 4.7 and 5.1 years). Even if changes in the level of sleep pressure in our young subjects existed, we believe these did not affect the topographical power distribution for several reasons: 1) Comparing baseline and sleep after sleep deprivation Finelli et al. found a very similar topographical power distribution in adult subjects (Finelli et al., 2001a). 2) We examined a later time window of sleep and found a

similar EEG power distribution as in early sleep (see Results). Recordings in post-pubertal females were scheduled to the follicular phase. This approach was chosen to prevent the variation of sleep EEG activity markers (e.g. spindle activity) as a function of the menstrual cycle phase (Driver et al., 1996).

EEG recording

All-night sleep electroencephalography (EEG), electrooculogram (EOG) and electromyogram (EMG) were recorded in 41 subjects (age range 8.7-19.4y, 23 males) in the sleep laboratory of the University Children's Hospital Zurich (Switzerland). Of the 41 subjects, three pairs were siblings (6 subjects). Fourteen children (age range 2.4-8.0y, 6 males) were recorded at home (in Providence, RI, USA). Of this sample, one pair were siblings, and two time four children were siblings (two boys were monozygotic twins). In total, 55 subjects were recorded. We excluded two subjects from the analysis: one girl because the recording did not taking place during the appropriate menstrual phase; one subject because bedtime reports identified the individual as an extreme short sleeper. Thus, 53 subjects were included in the analysis unless explicitly stated. All participants were monitored during one night using high density (HD) sleep EEG (Electrical Geodesics Sensor Net for long term monitoring, 128 channels, referenced to a vertex electrode for direct visualization and to the average across all channels for data analysis; details follow). The nets were adjusted to the vertex, and the cap electrodes were filled with gel electrolyte. The use of gel ensured the maintenance of good signals even after 8-10 hours (Landsness et al., 2009; Maatta et al., 2010). Impedances were measured after applying the gel and at the beginning of the recording. Electrode impedances were set below 50 kΩ. In one subject, the electrodes were removed after five hours due to discomfort. The sleep episode of each subject was scheduled according to individual reported bedtime. Subjects were awakened in the morning to allow school or job participation, resulting in variable bedtimes and rise times (variables not further examined).

Preprocessing

Data were sampled at 500 Hz (0.01 - 200 Hz), and referenced to the vertex (Cz). Then the EEG was band-pass filtered (0.5 - 50 Hz) and

down-sampled to 128 Hz. In one subject with low frequency sweating artifacts, a 0.75 Hz high-pass filter was used. Artifacts were rejected on a 20-s basis after visual inspection and if power exceeded a threshold based on a mean power value in the 0.75-4.5 Hz and 20-30 Hz bands (Huber et al., 2000). Poor quality EEG channels were excluded (on average 4 channels per subject). In total, 11.1% of the epochs were rejected (no significant age-related differences, two subjects were excluded from this age comparison, because of poor signal during more than an hour). All further analyses are based on re-referenced data: For every EEG sample the value of each channel was divided by the average value across all 109 channels above the ears that were not excluded (only good quality channels).

The EEG was visually scored for sleep stages (20-s epochs, C3A2 or C4A1) based on AASM standard criteria (Iber et al., 2007). Non rapid eye movement (NREM) sleep episodes were defined according to standard criteria (Rechtschaffen and Kales, 1968; Feinberg and Floyd, 1979) and adapted because of frequently occurring “skipped” REM sleep after the first NREM sleep episode (“skipped” REM occurred in 26% of the nights). Specifically, similar to (Jenni and Carskadon, 2004) and (Kurth et al., 2010b), we manually subdivided the first NREM sleep episode if a) the duration of the first NREM episode exceeded 120 minutes, and if b) stage 3 sleep in the first NREM episode was interrupted for at least 12 continuous minutes of stage 1 sleep, stage 2 sleep, wakefulness, or movement time. If both criteria were met, the first NREM sleep episode was subdivided at the lowest SWA. If the criteria were not met but the hypnogram and the SWA time course appeared as "skipped REM" (i.e. apparent interruption of sleep stage 3, obvious drop in SWA) we subdivided the cycle manually (11% of the recordings). In one subject, only the first 7 hours of data were included in the analysis due to poor signals. In another subject, the EEG signal was affected by artifacts in the frequency bands 14.5-15.5 Hz and 22-23 Hz (bands omitted for the analysis).

Spectral analysis

For qualitative exploration, spectral analysis was performed for all channels (fast Fourier transform routine, Hanning window, 20-s

epochs (averages of five 4-s epochs), frequency resolution of 0.25 Hz). The 20-s spectral power values were then averaged for a certain time window. Comparisons in EEG power spectra were assessed by ANOVA (one-way) with factor ‘age group’. When the ANOVA reached significance we performed post hoc Scheffé's test for multiple comparisons (significance at the 5% level). The lowest frequency bins (< 1Hz) were excluded from the analysis because of possible interactions with the high pass filter at 0.5 Hz (see Preprocessing). Spectral data was analyzed up to 25 Hz. Based on the spectral profile (Figure 2), subsequent analyses were restricted to specific (commonly used) frequency bands including slow waves (1-4.5 Hz), theta (4.75-7.75 Hz), alpha (8-9.75 Hz), sigma (10-15 Hz), and beta (20-25 Hz).

Power analysis and statistics

For further investigation, power maps were calculated for the defined frequency bands for all channels. EEG power for each electrode within a map was normalized to the average across the map. In order to further assess the age-related changes in topography along the postero-anterior axis, we compared the location of maximal power across five selected clusters of electrodes (Figure 4; see supplemental Figure S3 for more details). For each subject, the electrode with maximal power across all electrodes included in the five clusters was identified. The number of the cluster (from 1 to 5) which contained this electrode defined the Region Index (from 1 to 5). Thus, in each subject, the Region Index reflected the approximate location of maximal power for a defined frequency band. Next, individual region indices were averaged for each age group and statistically compared with ANOVA (two-way, factors ‘age group’ [see Results] and 'time' [early and late sleep, for details see Figure 2]) followed by Scheffé's multiple comparison test (significance at the 5% level). To account for differences in sleep episode durations, we included the first 60 min of NREM sleep stages 2 and 3 (if no additional information is given in the text). Data variability is described as SEs. All analyses were performed with the software package MATLAB (Math Works).

Anatomical localization of electrodes was verified in 35 subjects (8.7-19.4y) using magnetic MRI and the positioning system Northern Digital Inc.'s Navigation System. Electrodes were digitized and co-registered with the subject's MRI using SofTaxic Optic (EMS, Bologna, Italy) and the 3D optical digitizer (NDI, Polaris Vicra). T1-weighted anatomical images were obtained on a 3T scanner, a General Electrics Signa HDx. MR scans were collected in the axial plane (repetition time 8.928 ms, echo time 3.496 ms, flip angle 13º, final resolution 0.94 x 0.94 x 1.2mm).

Results

For the assessment of age-dependent changes, we subdivided the study population into the following six age groups: preschoolers (2-5 years), school-age children (5-8, 8-11, 11-14 years), young adolescents (14-17 years) and older adolescents (17-20 years). First, we examined visually scored sleep variables to evaluate the sleep quality of the sample (Table 1). Sleep quality was good, i.e. rather short sleep latency and high sleep efficiency, and we did not find a difference between home and lab recordings for sleep latency and efficiency (unpaired t-tests, $p>0.05$). Sleep efficiency (group means from 84% to 91%) and sleep latency (ranging from 13 to 25 min) were consistent with laboratory-based measures reported from other studies (Mason et al., 2008). We did not observe any age effect on sleep latency and efficiency, which is in agreement with earlier findings (Ohayon et al., 2004). However, we found age-dependent changes for REM sleep: in line with earlier findings (Roffwarg et al., 1966a), increasing age was associated with a significant decline in REM sleep (2-5 year olds: 28%; groups including 8-20 year olds: 18-20%; see Table 1 for details). In summary, we found minor age-related changes in sleep architecture based on standard visual scoring.

age groups	**2-5y**		**5-8y**		**8-11y**		**11-14y**		**14-17y**		**17-20y**	
n (total 51)	8		5		12		9		12		5	
Sleep variables												
time in bed (min)	651.6	± 18.4	579.9	± 17.4	528.0	± 9.9	523.6	± 13.4	469.6	± 13.8	399.6	± 24.8
total sleep time (min)	581.9	± 10.1	520.6	± 16.7	444.5	± 12.3	469.5	± 17.1	429.2	± 17.7	363.5	± 15.1
sleep efficiency (%)	89.6	± 2.0	89.8	± 1.5	84.4	± 2.7	89.7	± 2.4	91.1	± 1.8	91.4	± 2.1
sleep latency (min)	24.8	± 7.3	18.5	± 4.3	24.8	± 2.5	22.7	± 5.4	20.6	± 3.6	13.6	± 3.2
Waking after sleep onset (min)	46.5	± 14.2	43.0	± 9.9	62.1	± 13.8	35.4	± 8.6	23.5	± 5.0	25.7	± 9.0
stage 1 (%)	4.2	± 0.3	3.5	± 0.8	7.3	± 0.9	7.5	± 1.0	8.6	± 1.3	5.4	± 1.6
stage 2 (%)	47.8	± 2.2	51.7	± 2.2	46.5	± 2.4	46.4	± 2.4	52.4	± 1.3	57.6	± 2.1
SWS (%)	20.4	± 1.2	20.0	± 1.0	27.3	± 2.9	26.4	± 2.2	20.7	± 1.3	17.8	± 3.4
REM sleep (%)	**a** 27.6	± 1.3	24.8	± 1.1	**b** 18.8	± 1.2	**b** 19.7	± 1.1	**b** 18.3	± 1.4	**b** 19.2	± 1.4
Sleep cycle duration (min)	89.8	± 3.4	87.0	± 3.0	94.2	± 5.3	92.7	± 3.9	96.6	± 3.5	95.4	± 3.4

<Table 1. *Sleep variables (mean±SE) of all subjects with at least 4.5 hours of recorded sleep time and 4 sleep cycles subdivided into age groups (2.4-19.4y, n=51). 'Sleep efficiency', total sleep time expressed as a percentage of time in bed; 'sleep latency', latency to the first occurrence of stage 2 sleep; 'waking after sleep onset', expressed in minutes; sleep stages are expressed as a percentage of total sleep time; 'SWS', slow-wave sleep as stage 3 sleep; 'REM' sleep, rapid eye movement sleep. 'Sleep cycle duration' includes only the first 4 sleep cycles. Because subjects were awoken in the morning, the last sleep cycle could not always be completed. Thus we applied two additional criteria for the analysis of cycle duration: In subjects with only 4 cycles, but with a missing REM sleep episode in the last cycle, the last cycle was excluded from the comparison (n=6). Moreover, only sleep cycles containing a minimum of 75 epochs of NREM sleep were included. Sleep variables 'time in bed' and 'total sleep time' were excluded from group comparisons, because sleep was restricted in the morning (for details see Methods). A one-way ANOVA with factor age group was significant for stage 1 and stage 2 sleep, SWS and REM sleep (all p<0.05, F=0.6-7.7, df=5). Post-hoc Scheffé tests revealed a significant group difference for REM sleep as indicated by letters (p<0.05). Age groups with different letters differ significantly from each other.*

Age-related changes in the spectral profile of the sleep EEG

Visual inspection of the sleep EEG revealed distinct age-related aspects. As expected, the three individual representative examples of different ages showed prominent slow waves (red marks) during deep sleep, however, their expression was variable (Fig. 1). Most pronounced slow waves were found in school age children (Fig. 1, middle panel). The sleep EEG of the preschooler showed characteristic theta oscillations (Fig. 1, upper panel, blue marks).

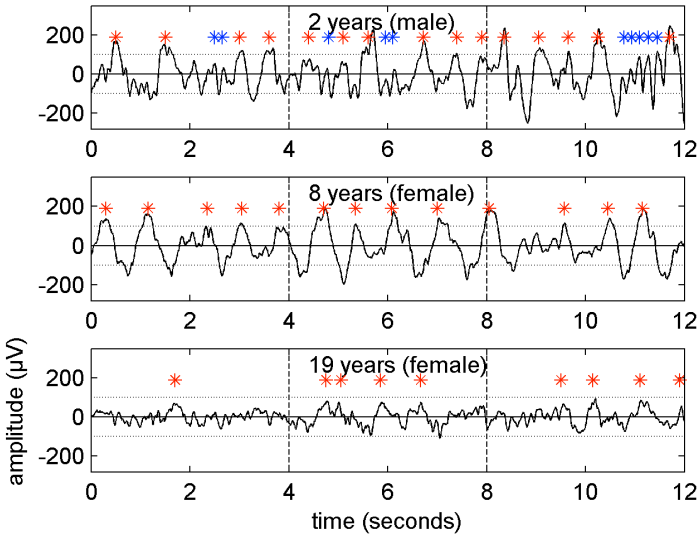
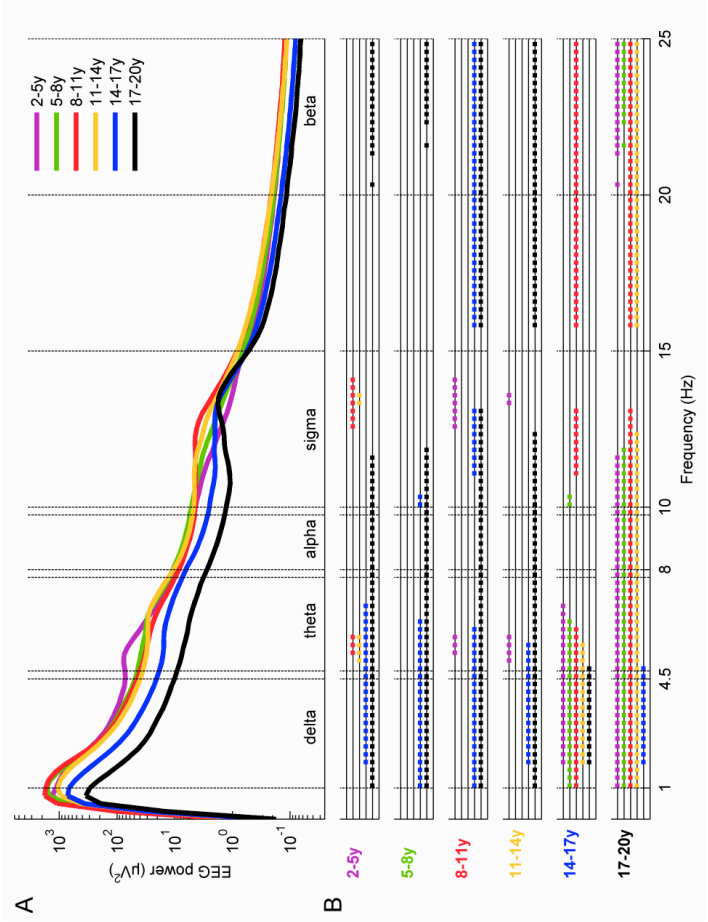


Figure 1. *Tracings of slow-wave sleep for different ages. Twelve second EEG segments of slow-wave sleep in the first sleep cycle in three subjects of different ages (C3A2 derivation). Red stars indicate slow waves, blue stars theta waves.*

Next, we quantified age-related changes in the sleep EEG by spectral analysis and observed the classical spectral profile of the NREM sleep EEG (Fig. 2A). All age groups expressed the greatest power in the SWA range (1-4.5Hz). A second and third local maximum was found in the theta (4.75-7.75Hz) and sigma (10-15Hz) frequency ranges of the power distribution. In general, the most pronounced group differences in the first 60 minutes of NREM sleep were present in the low frequencies (SWA and theta, Fig. 2B). Fewer group differences were found in the alpha frequency range, while in the high sigma range no differences between age groups were observed. Differences in the beta frequency range were similar to those observed in the alpha frequency band. We performed the same analysis for a later time window during the sleep period and found a similar pattern of age group differences (supplemental Figure S1). We limited subsequent analyses to specific frequency ranges (SWA,

theta, alpha, sigma, beta) as defined by the spectral profile across all age groups (Fig. 2A).



>Figure 2. *A) EEG power spectra during early NREM sleep. Average of all electrodes of the first 60 min NREM sleep stages 2 and 3 in 6 age groups (purple 2-5y, green 5-8y, red 8-11y, yellow 11-14y, blue 14-17y, black 17-20y; n=53). Significant ANOVAs (see Methods, F=2.5-25.7, df=5) were followed by group-wise comparisons as indicated in B): Post-hoc Scheffé testing calculated for each 0.25 Hz bin and significant group differences indicated as dots (p<0.05). Comparisons are color coded: Groups labeled on the ordinate*

were compared with color-coded groups for each particular frequency bin. A later time window of sleep showed a similar pattern of age group differences (supplemental material).

Sleep EEG topography from early childhood through late adolescence

We next examined the topographical distribution of EEG power of the selected frequency ranges in the defined age groups (Fig. 3). All frequency ranges showed specific distribution patterns with maximal and minimal power expressions. These maxima and minima are indicated next to each plot and illustrate the overall time course of EEG power across age. In agreement with earlier findings (e.g. ((Campbell and Feinberg, 2009)), we found a significant power loss with an onset around age 10 years for all frequency ranges. Only the slow wave and sigma frequency ranges showed an increasing trend within the first decade of life.

Given the overall time course of EEG power across ages, we normalized each map to the average value across the map to assess age-related changes in topography. We found marked changes in the SWA range: in the two youngest groups, SWA was prevalent over the occipital lobe, while power showed a forward shift in the subsequent age groups. In contrast, other frequency ranges revealed only minor changes across age. Sigma power showed a prefrontal maximum that elongated towards central and occipital regions in the oldest group. A subdivision of the sigma range into slow and fast spindles (Werth et al., 1997) revealed the latter accounted for the central relative increase, while slow spindles extended more towards frontal and occipital regions in the oldest group (data not shown). To quantify the variability of the topography across subjects we calculated the relative error of the mean for each electrode (supplemental Figure S2). The variability across subjects was low (with the exception of few electrodes). Variability was largest in the beta frequency range indicating that these topographical distributions should be interpreted with caution. We also assessed the stability of topographical patterns across the night. The late sleep time window showed similar topographical distribution of EEG power in all frequency ranges and age groups (data not shown).

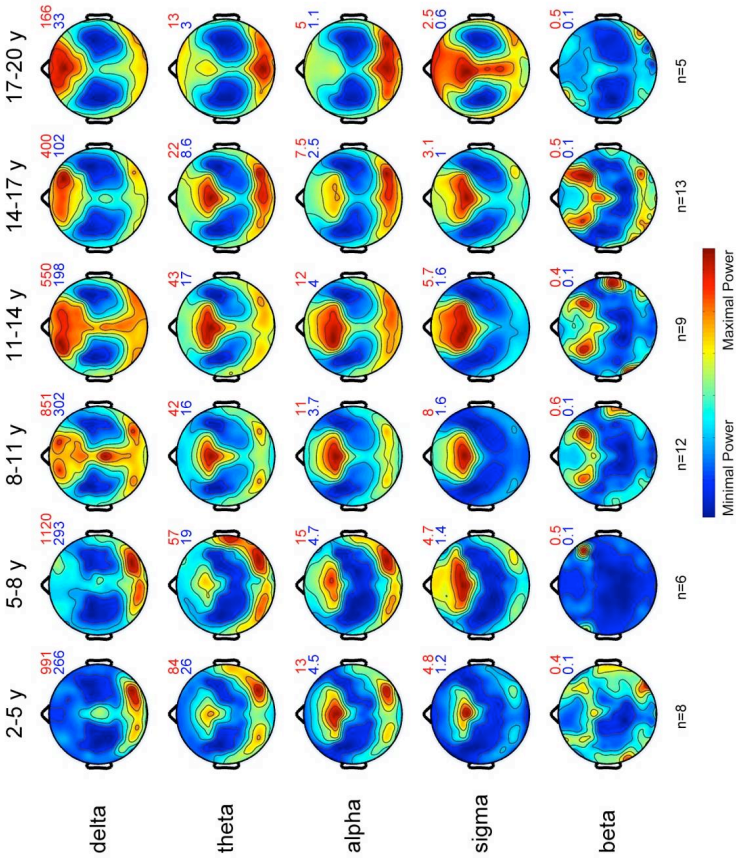


Figure 3. *Maps of EEG power during NREM sleep. Topographical distribution of NREM sleep EEG power for the defined age groups and frequency ranges (n=53). Maps are based on 109 derivations from the first 60 min of NREM sleep stages 2 and 3. Maps were normalized for each individual and then averaged for each age group. Values are color coded (maxima in red, minima in blue) and plotted on the planar projection of the hemispheric scalp model. To optimize contrast, each map was proportionally scaled and values in between the electrodes were interpolated. At the top right of the maps numbers indicate maxima and minima (in square microvolts) for each plot.*

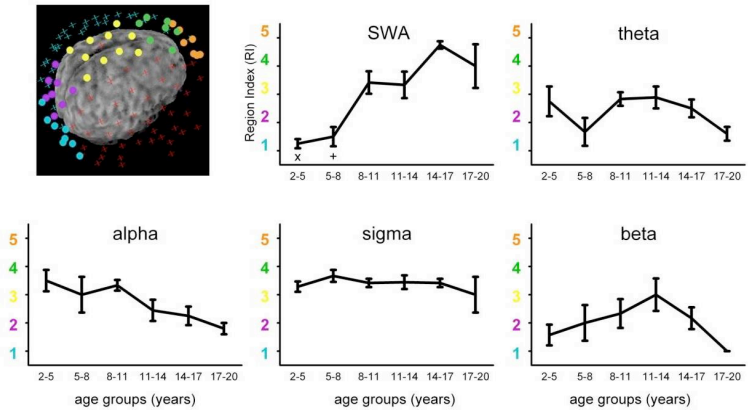


Figure 4. *Region index for selected frequency bands. Five cortical subregions along the inion-nasion axis are illustrated as clusters of coloured electrodes (blue= Region Index (RI) 1, purple= RI2, yellow= RI3, green= RI4, orange= RI5; the remaining electrodes are illustrated by turquoise and red crosses). For each subject, the location of maximal power over all clusters was determined. Depending on the cluster in which that maximal power value occurred, a value (RI) from 1 to 5 was given. The electrodes were digitized and co-registered with the subject's magnetic resonance images. RIs for age group are presented for the selected frequency bands. Two-way ANOVA with factors age group and time (early or late sleep, see legend of Fig. 2C) showed significant effects for age group in the SWA range ($p<0.05$, $F=13.8$, $df=5$), theta ($p<0.05$, $F=4.5$, $df=5$) and alpha ($p<0.05$, $F=7.2$, $df=5$) band, while the factor time was not significant and no interaction was found. Post-hoc testing revealed a significant main effect for age group for the SWA range (Scheffé tests, $p<0.05$; x: 2-5y differ significantly from 8-11y, 11-14y, 14-17y and 17-20y; +: 5-8y differ significantly from 8-11y, 14-17y and 17-20y).*

Maximal expression of sleep EEG power along the postero-anterior axis

For a statistical comparison of the shift of EEG power along the postero-anterior axis, we limited the analysis to five regions of interest (Fig. 4). A number ranging from 1 (most posterior) to 5 (most anterior) indicated the region of interest in which the power maxima for a certain frequency range and age group occurred (region index, RI,

see Methods for more details). We found significant age-dependent effects only for the SWA range. The RI of SWA showed a significant increase with age reflecting the shift of the power maxima from posterior to anterior cortical regions (from 1.3±0.2 in the youngest to 4.0±0.8 in the oldest group) with the most apparent change below the age of 11 years (Fig. 4). Other frequency ranges did not exhibit significant age-related changes of RI. We again tested this observation for stability across the night and found the similar age-related postero-anterior shift of SWA maxima in late sleep (and no age-related changes in other frequency ranges, data not shown).

For an anatomical localization of the RI, we co-registered the electrodes with individual T1-weighted magnetic resonance images (see Methods for more details). An orthogonal projection of the electrode onto the cortex showed the SWA maxima in preschoolers over the occipital lobe (Lingual Gyrus) and in older adolescents over the frontal lobe (Medial Frontal Gyrus) (Table 2).

	Electrode cluster	Talairach coordinates (mean ± SE)			Anatomical localization	Range
	Region Index (1-5), F/O Index	X	Y	Z	Lobe, Brodmann Area (BA)	(mm)
Most posterior	1	-3 ± 2	-92 ± 3	-14 ± 3	Occipital Lobe, Lingual Gyrus, BA 18	1
	1, O	-4 ± 2	-85 ± 2	33 ± 3	Occipital Lobe, Cuneus, BA 19	0
	2	-4 ± 2	-62 ± 2	58 ± 2	Parietal Lobe, Precuneus, BA 7	1
	2	-5 ± 2	-44 ± 2	65 ± 2	Parietal Lobe, Postcentral Gyrus, BA 5	0
	3	-4 ± 2	-9 ± 3	68 ± 2	Frontal Lobe, Medial Frontal Gyrus, BA 6	1
	3	1 ± 2	9 ± 2	63 ± 3	Frontal Lobe, Superior Frontal Gyrus, BA 6	4
	3	1 ± 1	41 ± 2	51 ± 1	Frontal Lobe, Superior Frontal Gyrus, BA 8	3
	4, F	2 ± 1	63 ± 1	24 ± 2	Frontal Lobe, Medial Frontal Gyrus, BA 10	2
	5, F	2 ± 1	67 ± 1	9 ± 2	Frontal Lobe, Medial Frontal Gyrus, BA 10	2
	5, F	3 ± 1	66 ± 0	-3 ± 1	Frontal Lobe, Medial Frontal Gyrus, BA 10	1
Most anterior	5	0 ± 1	56 ± 1	-17 ± 1	Frontal Lobe, Medial Frontal Gyrus, BA 11	2

Table 2. *Anatomical localization of the electrodes along the antero-posterior axis. Electrodes were digitized and co-registered with the subject's magnetic images. Electrodes of the Region Index (RI; see Figure 4) and Frontal/Occipital index (F/O; see Figure 5) are included. Average Talairach coordinates (±SE) are indicated for 35 subjects. The corresponding lobe, gyrus, and Brodmann Area were detected using the Talairach Client (Lancaster et al., 1997; Lancaster et al., 2000). The range (in mm) refers to the distance of the nearest grey matter area with respect to the coordinates.*

Figure 5 illustrates the ratio of EEG power of frontal (F) to occipital (O) electrodes and quantifies the age-related shift of maximal expression of SWA along the postero-anterior axis. The F/O-ratio of SWA correlated strongly with age (R2=0.67; p<0.0001) and remained stable across the night (R2=0.66 for late sleep; p<0.0001).

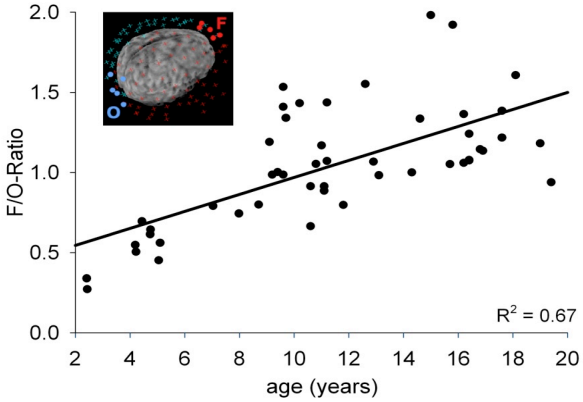


Figure 5. *F/O ratio across age. SWA within a cluster of five electrodes in the frontal region (F) was averaged and divided by the value of five occipital electrodes (O). The cluster of electrodes included in the F/O-ratio is illustrated for an older adolescent (inset). The SWA F/O-ratio correlated significantly with age (p<0.001).*

Discussion

This study examined sleep EEG topography from early childhood through adolescence. The main finding shows that the location with maximal SWA undergoes a shift from posterior to anterior regions across childhood and adolescence. None of the other frequency ranges demonstrated similar age-related spatial changes. This finding fundamentally expands previous knowledge about the maturation of the sleep EEG, because no comprehensive examination exists so far regarding i) the first two decades of life and ii) the spatial resolution that allows for the description of regional differences. According to anatomical (Von Economo, 1929), neuroimaging (e.g. MRI grey

matter thickness; (Shaw et al., 2008)) and behavioural studies (e.g. cognitive functions; (Luna and Sweeney, 2004)) cortical maturation follows a similar time course along the postero-anterior axis. The parallel time course of cortical maturation and sleep SWA may suggest that SWA reflects cortical plasticity during development.

Sleep depth exhibits prominent changes in the first two decades of life (Jenni and Carskadon, 2004; Feinberg and Campbell, 2010), and such changes in sleep depth are best characterized by EEG SWA during NREM sleep. Longitudinal and cross-sectional studies show an inverted U-shape time course of SWA, i.e. an increase of SWA until puberty, followed by a decrease during adolescence (Feinberg, 1982; Gaudreau et al., 2001; Jenni and Carskadon, 2004; Campbell and Feinberg, 2009). Campbell and Feinberg alluded to the similarity of the time course of synapse density and SWA (Feinberg, 1982; Campbell and Feinberg, 2009), proposing the decrease in SWA observed during adolescence reflects the decrease or pruning of synapses (Campbell and Feinberg, 2009; Feinberg and Campbell, 2010). Our data support this notion: we also observed the same overall decrease of SWA during adolescence. Recently, Vyazovskiy et al. used multiunit recordings in the rat and showed increased synaptic strength allows for faster synchronization of cortical activity across neurons, resulting in larger amplitude slow waves as observed with scalp EEG recordings (Vyazovskiy et al., 2009). This observation provides a mechanistic explanation for the parallel time course of synapse density and overall SWA (Huttenlocher, 1979; Feinberg and Campbell, 2010; Kurth et al., 2010b). Moreover, more and/or stronger synapses lead to increased energy consumption (Attwell and Laughlin, 2001). This finding might explain the similar time course of glucose usage, which shows a similar inverted U-shape curve as SWA and synaptic density during the first two decades of life (Chugani, 1987). If synaptic strength is a key determinant for the level of synchronization in cortical networks, frequency ranges other than SWA should exhibit age-dependent changes. In fact, the decrease of sleep EEG power across adolescence is not limited to the SWA frequency range, but also includes the theta range (Campbell and Feinberg, 2009). Again, we confirm this observation in our subject population. Finally, the effects of changes in the level of

synchronization may not be limited to sleep. EEG recordings during wakefulness showed a similar inverted U-shape time course of EEG power in the alpha frequency range (Gasser et al., 1988).

Previous sleep EEG studies during childhood and adolescence were restricted only to few derivations (Feinberg, 1982; Gaudreau et al., 2001; Jenni and Carskadon, 2004; Campbell and Feinberg, 2009). In contrast to former studies about the maturation of the sleep EEG using a limited number of electrodes, we successfully performed HD EEG recordings (with up to 128 electrodes) in children and adolescents to map changes in the sleep EEG with high spatial resolution. We believe high spatial resolution is important for two reasons. First, although sleep was considered a global phenomenon for many decades, a growing number of studies in recent years indicate otherwise – that is, sleep is a localized process. For example, several authors found a frontal predominance of SWA in adults (Werth et al., 1997a; Cajochen et al., 1999; Finelli et al., 2001b; Huber et al., 2004). Second, anatomical, neuroimaging and behavioural studies report regional cortical maturation, which follows a tightly programmed course (Huttenlocher and Dabholkar, 1997; Luna and Sweeney, 2004; Shaw et al., 2008).

Similar to reports from adults, we observed regional differences in the distribution of EEG power for all classical frequency ranges. However, only the topography of EEG power in the SWA frequency range exhibited clear age-dependent changes. Thus, the most striking observation of our analysis was the substantial shift of the predominance of SWA during childhood and adolescence. Our anatomical localization of maximal occurrence of SWA revealed that first the maxima occurs over occipital lobe in preschoolers, followed by parietal regions and posterior frontal lobe in school-age children (from 8 to 14y) and, finally, the SWA maxima spreads to frontal lobes during adolescence. Young and older adolescents (after about 14 years of age) present a frontal predominance of SWA as found in adults (Figure 3) (Werth et al., 1996b; Cajochen et al., 1999; Finelli et al., 2001b). However, the frontal predominance of SWA in both younger and older adolescents is not yet as pronounced as in adults (Huber et al., 2004). These findings indicate that SWA topography

undergoes maturational changes from childhood to late adolescence. The timing and the location of these changes in SWA topography are closely paralleled by anatomical and behavioural developmental changes. Already in the 1920s Von Economo described cytoarchitonical changes spreading from back to front (Von Economo, 1929). Huttenlocher and Dabholkar also used post-mortem samples to quantify synaptic densities of different cortical regions across age (Huttenlocher and Dabholkar, 1997). According to their study, all areas showed an inverted U-shape time course of synapse density. However, the age at which peak synaptic density was reached varied considerably: the occipital cortex was first and the frontal cortex was last. More recently such regional changes in cortical maturation have been tracked by MRI. Several studies found primary motor and sensory areas reach peak cortical thickness first, followed by secondary and association areas and the frontal lobe matures last (Giedd, 2004; Sowell et al., 2004; Shaw et al., 2008). According to these imaging studies, cortical maturation starts in occipital poles and sensorimotor areas and spreads rostrally over the frontal cortex and caudally over parietal and then temporal cortex (Brecelj, 2003; Shaw et al., 2008). Finally, strong evidence for a local maturation of the cortex from behavioural observations exists. For example, executive functions, which are strongly dependent on frontal cortices (Tau and Peterson, 2010), are not fully mature until late adolescence (Spear, 2000; Luna and Sweeney, 2004). On the other hand, visual acuity, a task predominantly performed by primary visual cortex (occipital lobe) matures in the first years of life (Teller, 1981).

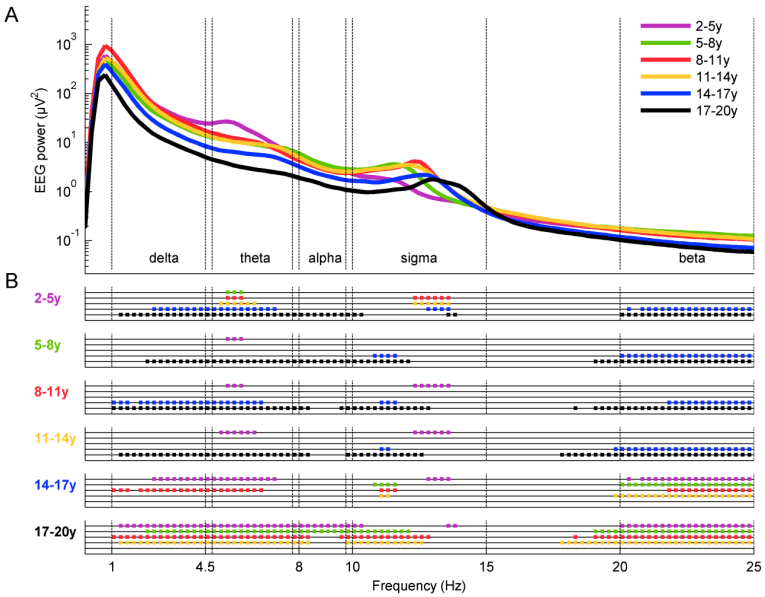
In summary, SWA may reflect not only global changes in synapse density but also mirror the regional aspects of cortical maturation. A major factor underlying all such maturational changes is the age-dependent alteration of synaptic density. Changes in cortical volume and thickness across childhood and adolescence are attributed to the alterations in synaptic density and linked to the maturation of cognitive functions (e.g. (Sowell et al., 2004)). Changes in synaptic density lead to a change in the level of synchronization of the activity among neurons, which on the scalp is reflected by changes in the amplitude of rhythmic activities (Vyazovskiy et al., 2009). While such a mechanism would explain the changes in SWA topography, we

cannot exclude the possibility that additional factors may play a role in the relationship between cortical maturation and sleep SWA. For instance, a reduction in synapse number could be accompanied by a reduction in glial cells, resulting for example in changes of cortical thickness and volume (Paus, 2005). An association between glial cell activity and sleep SWA was recently proposed (Halassa et al., 2009).

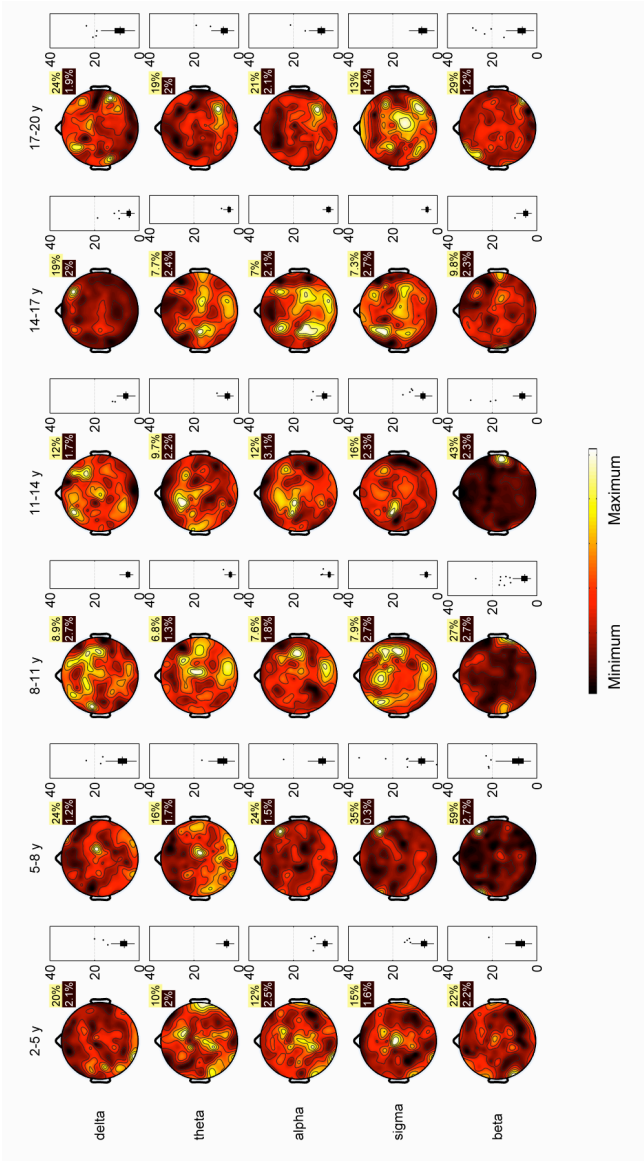
Whatever factors during cortical maturation are responsible for the significant topographical changes of SWA, our results suggest this relationship is frequency specific, because none of the other frequency ranges showed a similar spatial evolution of topography. This finding is in contrast to the global time course of EEG power across childhood and adolescence reported for several frequency ranges (Figure 2 and 3; (Campbell and Feinberg, 2009)). Recent hypotheses about the function of sleep include sleep slow waves as the key electrophysiological feature during NREM sleep (Tononi and Cirelli, 2006; Diekelmann and Born, 2010). One of these hypotheses, the synaptic homeostasis hypothesis by Tononi and Cirelli (Tononi and Cirelli, 2006), directly relates sleep slow waves to synaptic plasticity. The hypothesis proposes that the activity of sleep slow waves directly reflects synaptic strength. Thus, the more a cortical network undergoes synaptic potentiation due to learning processes during wakefulness (Whitlock et al., 2006), the more SWA is expressed during subsequent sleep. The synaptic homeostasis hypothesis is supported by both electrophysiological and molecular data (Tononi and Cirelli, 2006). For instance, a major molecular marker of cortical plasticity, brain-derived neurotrophic factor (BDNF), is causally related to SWA (Faraguna et al., 2008). Blockage of BDNF by direct infusion of Anti-BDNF results in a subsequent reduction of SWA. In contrast, local infusion of BDNF results in a corresponding increase of SWA. Also, experiments in humans support the relationship between cortical plasticity and sleep SWA. For example, Huber and colleagues reported the induction of plastic changes either during a learning task or directly by transcranial magnetic stimulation led to a local increase of SWA during subsequent sleep (Huber et al., 2004; Huber et al., 2008).

The current results further support a close relationship between cortical plasticity and sleep slow waves and suggest SWA may be used to follow cortical maturation during sensitive periods such as adolescence (Paus et al., 2008). “Overpruning” during adolescence has been linked to schizophrenia, mood disorders (Saugstad, 1994), autism, and mental retardation (Tessier and Broadie, 2009). Thus, the monitoring of synaptic remodeling during childhood and adolescence may increase our understanding of the pathophysiology of such disorders, lead to early diagnosis and guide potential therapies (Woo and Crowell, 2005). While MRI allows for a relative easy, non-invasive method for tracking anatomical changes, the relationship between anatomy and function are not trivial. The literature suggesting MRI parameters predict behaviour is contradictory. For example, studies reveal experience-dependent alterations of grey matter in specific areas (Draganski et al., 2004; Boyke et al., 2008; Jancke et al., 2009), however, the physiological or cellular basis of these changes is unclear (Jancke et al., 2009). Only a thorough test of many cognitive functions using functional MRI and extensive behavioural testing may allow tracking of cortical functioning during development. Electroencephalography, i.e. the recording of the electrical activity of the brain, may provide a more direct assessment of cortical functioning. In particular, the activity during deep sleep permits a unique opportunity to quantify cortical network properties because the network during this sleep stage is - in comparison to waking - not disturbed by active behaviour and to a lesser amount responding to external stimuli. The cortical activity during deep sleep is dominated by sleep SWA, and recent studies indicate SWA reflects not only synaptic density (number) but also synaptic strength (e.g., levels of AMPA receptors per synapse) and synaptic efficacy (magnitude of the physiological effects, e.g., postsynaptic currents (Tononi and Cirelli, 2006). We propose the regional expression of SWA across cortical maturation may be a promising marker for plastic changes during childhood and adolescence. Thus, making sleep SWA a powerful tool to investigate cortical maturation in health and disease.

Supplementum

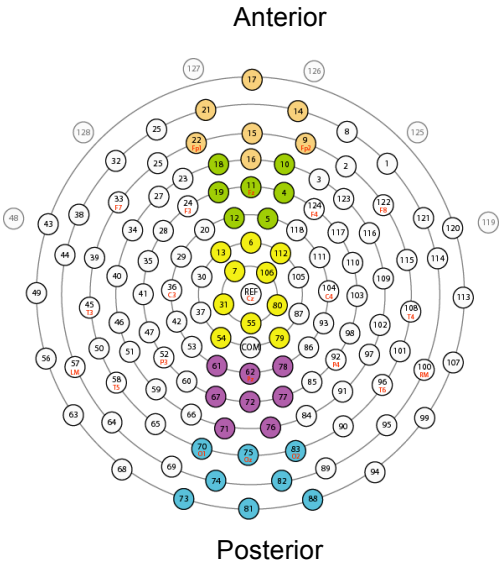


Supplementary Figure S1. *EEG power spectra during late NREM sleep. A. EEG power spectra are presented for 6 age groups (purple 2-5y, green 5-8y, red 8-11y, yellow 11-14y, blue 14-17y, black 17-20y). Data include the latest possible 60 minutes of common NREM sleep across all subjects (i.e. 133rd - 193rd min of artifact-free NREM sleep stages 2 and 3; average of all electrodes). Data includes 52 subjects, one subject was excluded, because the electrodes were removed after five hours due to discomfort. Significance in ANOVAs (see Methods, F=2.8-21.0, df=5) were followed by group-wise comparisons as indicated in B: Post-hoc Scheffé testing calculated for each 0.25 Hz bin, significant group differences indicated as dots (p<0.05). Comparisons are color coded: Groups labeled on the ordinate were compared with color-coded groups for each particular frequency bin.*



Supplementary Figure S2. *Topographical distribution of the relative variability of EEG power. The standard error of the mean power is presented as a percentage of the mean*

power for each electrode, in all age groups and frequency bands. Maps are based on 109 derivations from the first 60 min of NREM sleep stages 2 and 3. Values are color coded (maxima in white/yellow, minima in brown) and plotted on the planar projection of the hemispheric scalp model. To optimize contrast, each map was proportionally scaled and values in between the electrodes were interpolated. At the top right of the maps numbers indicate maxima and minima (in %). Boxplots include values of 109 electrodes with central horizontal line representing the median, edges of the box are the 25th and 75th percentiles, the whiskers extend to the most extreme data points not considered outliers and outliers are represented as individual dots. The ordinate is limited to 0-25% for visualization purposes. The three outliers not presented are: 58% and 59% in 5-8y, 43% in 11-14y in the beta frequency band.



Cluster Index	Electrode numbers
5	9 14 15 16 17 21 22
4	4 5 10 11 12 18 19
3	6 7 13 31 54 55 79 80 106 112
2	61 62 67 71 72 76 77 78
1	70 73 74 75 81 82 83 88
frontal	10 11 15 16 18
occipital	71 74 75 76 82
midline	17 15 16 11 6 Cz 55 62 72 75 81

Supplementary Figure S3. *Electrode information. The high density electrode net layout is shown from top view, labeled with the numbers adapted from the Electrical Geodesics, Inc. layout. Colours refer to the five selected clusters of electrodes (details in the manuscript and in Figure 4), the table lists the electrode numbers of the clusters, and the electrodes used for the analysis of a frontal and an occipital cluster (see Figure 5). The electrodes termed 'midline' were used for anatomical electrode localisation (see Table 2). RM, LM: right and left mastoid. COM: special ground electrode (see Net Station Acquisition manual of Electrical Geodesics, Inc.).*

Research Part III: SWA as a tool

5

The sleep EEG topography in adolescents shows sex differences in language areas

Maya Ringli[1], Salomé Kurth[1], Oskar G. Jenni[1,2] and Reto Huber[1,2]

1) Child Development Center, Children's University Hospital Zurich, 8032 Zurich, Switzerland

2) Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland

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Abstract

The topography of sleep slow wave activity (SWA), a major characteristics of non-rapid eye movement (NREM) sleep, was recently shown to mirror the actual state of cortical maturation and functioning during development, expressing a pattern which is typical for a certain age range. We therefore examined if typical sex differences (in behaviour and brain structure) are reflected in sleep SWA of adolescents. Comparing slow-wave sleep of 22 age-matched subjects (11 boys and 11 girls, mean age 13.4 ± 3.9) during the first sixty minutes of NREM sleep revealed that females exhibited higher SWA over cortical areas bilaterally that are related to language function, whereas males showed increased SWA over the right prefrontal cortex, a region also involved in spatial abilities. We conclude that cortical areas, involved in functions in which one sex outperforms the other, may exhibit higher plasticity, which is reflected in increased sleep SWA. Thus, mapping sleep SWA may be a useful tool to uncover changes in cortical plasticity.

Introduction

Recently, the topographic distribution of slow wave activity (SWA; EEG power between 0.75 and 4.5 Hz), a major electrophysiological feature of non-rapid eye movement (NREM) sleep, was shown to parallel cortical maturation from childhood through adolescence (Kurth et al., 2010a). High-density EEG recordings in children and adolescents between 2 and 20 years of age showed that SWA exhibited a regional predominance, which was characteristic for a certain age range. When the location of maximal SWA was identified across age, a shift from posterior to anterior regions was observed, reaching frontal derivations during adolescence. Strikingly, the local SWA maxima paralleled the time course of cortical grey matter (Gogtay et al., 2004; Sowell et al., 2004) and behavioural maturation (Luna and Sweeney, 2004), indicating that SWA is a marker of cortical plasticity during development. This interpretation is in line with an increasing number of reports showing a direct relationship between sleep slow waves and plastic processes (Tononi and Cirelli, 2006; Vyazovskiy et al., 2009). Specifically, it has been recently hypothesized that wakefulness is associated with a net increase in synaptic strength that is homeostatically rebalanced during sleep. This close relationship was shown in various species using key markers of synaptc strength: In cortical slices the frequency and amplitude of miniature excitatory post-synaptic currents (Liu et al., 2010), in Drosophila melanogaster the protein levels of key components of central synapses (Bushey et al., 2011), in rats the slope of the local field potential evoked by electrical cortical stimulation (Vyazovskiy et al., 2008) and in humans the slope of transcranial magnetic stimulation evoked EEG responses (Bellina et al., 2008), all increased after wakefulness and decreased during sleep.

This functional relationship between sleep slow waves and synaptic strength may explain the close correspondence between cortical maturation, where massive changes in synaptic characteristics occur, and the topography of SWA. The undisturbed mapping of cortical activity during NREM sleep may thus represent a useful tool to

uncover anatomical differences during brain maturation. Since it is generally accepted that behavioural (e.g. language) and anatomical (e.g. grey matter volume) sex differences exist (Kimura, 2000; Luders et al., 2009), starting already during childhood (Plante et al., 2006; Burman et al., 2008; Porter et al., 2011), we examined whether these sexually dimorphic characteristics are also reflected in the topography of sleep SWA.

Materials and methods

Participants

In order to investigate sex differences in sleep EEG topography 11 boys (mean age 13.4 years, range: 8.7-19.4 years) and 11 age matched girls (mean age 13.4 years, range: 9.1-19.0 years) were selected from a previous study, where the topography of the sleep EEG frequency bands was studied across (Kurth et al., 2010a). All participants were right handed and reported no psychopathology, chronic diseases, sleep complaints or primary sleep disorders (including sleep disordered breathing and periodic limb movements of sleep), or current use of psychoactive agents or other medication. Written informed consent was obtained from the parents or from participants of full age after explanation of the study methods and aims. The procedures were approved by the local ethics committee, and the study was performed according to the Declaration of Helsinki. Sleep EEG recordings in females with menstruation were performed during the follicular phase, to prevent the variation of sleep EEG activity markers as a function of the menstrual cycle phase (Driver et al., 1996). One week prior to the study, participants were instructed to maintain regular sleep-wake schedules and to keep their alcohol and caffeine consumption on restricted levels. All participants were non-smokers. 24 hours before and during the whole study, they were asked to refrain from alcohol and medication. Compliance regarding sleep-wake schedules and alcohol consumption was verified with self-reported sleep logs and wrist motor actigraphy.

Recording and preprocessing of EEG data

All subjects were studied in the sleep laboratory of the University Children's Hospital Zurich (Zurich, Switzerland). All-night sleep EEG

recordings were performed by means of a 128-channel EEG amplifier (Electrical Geodesics Inc.). EEG recordings were sampled at 500Hz (0.01 - 200Hz) and referenced to the vertex (Cz), band-pass filtered between 0.5 and 50 Hz (except for one girl, where a 0.75 Hz low-pass filter was used to exclude low frequency sweating artefacts) and downsampled to 128Hz. Artefacts were rejected on a 20s basis after visual inspection and if power exceeded a threshold based on a mean power value in the 0.75-4.5 and 20-30Hz bands (Huber, Graf et al. 2000). After exclusion of EEG channels of insufficient quality (on average, two channels per subjects) the data was re-referenced to average reference.

Power analysis and statistics

For a detailed analysis of the sleep EEG, a spectral analysis of consecutive 20-s epochs (FFT routine, Hanning window, averages of five 4-s epochs) was performed for all channels. SWA was calculated as mean power in the range of 1-4.5 Hz during the first 60 minutes of NREM sleep stage 2 and 3 (Iber et al., 2007). EEG power for each electrode was normalized to the average of all electrodes.

In order to investigate differences in SWA topography between males and females, power maps were calculated for the slow wave frequency band for each group. EEG power for each electrode was normalized to the average across the map. To further investigate sex differences in SWA topography we defined clusters based on t-values of unpaired t-tests between the power maps. In our explorative approach we used a two-tailed probability of maximal 20% (t= 1.33, df= 20) to select the electrodes of interest. Mapping the t-values resulted in three clusters of electrodes (R1-R3) exhibiting a probability of maximal 20% or lower (figure 1c). Furthermore each cluster was defined to consist of a minimum of two electrodes. Two additional electrodes (one in each group) were therefore excluded from further analysis. Mean SWA for each cluster was compared between the two groups using unpaired t-tests.

Anatomical localization of electrodes was verified using MRI and the positioning software SofTaxic Optic (EMS Inc). Electrodes were digitized and co-registered with the subject's MRI (for details see (Kurth et al., 2010a)).

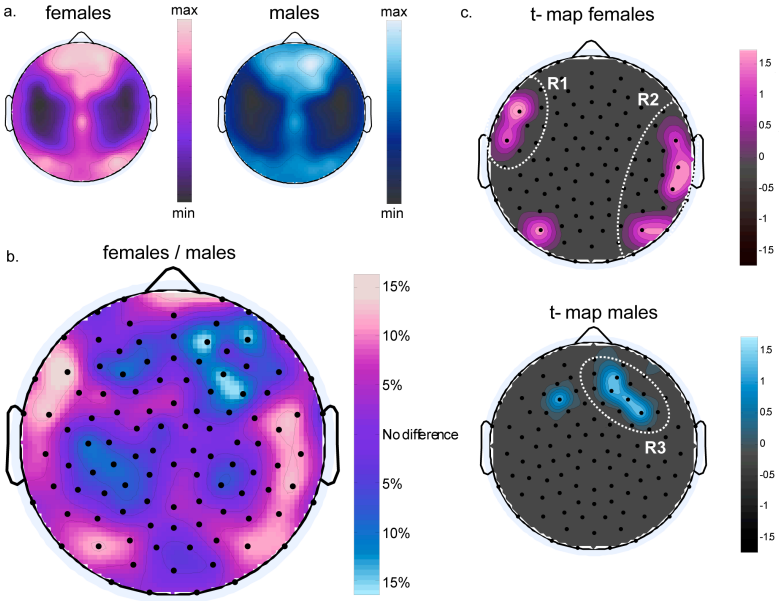
To assess anatomical sex differences in our sample grey matter thickness was estimated based on magnetic resonance images, using the Freesurfer software, version 4.5.0 for Mac OS 10.5.2 (http://surfer.nmr.mgh.harvard.edu) (for more details see (Buchmann et al., 2010)). Cortical thickness was measured below each digitized electrode perpendicularly to the surface outward to the pia mater. The thicknesses were measured as closely as possible to the average Talairach coordinates of each electrode (average error 3.7mm, SD 2.4mm) (Lancaster et al., 1997; Lancaster et al., 2000).

Results

In Figure 1a the topographical distribution of SWA during the first hour of NREM sleep is shown for females and males. Both groups showed a typical SWA topography i.e. a clear local maximum, sustained symmetry and bilateral temporal power minima. To explore potential sex differences in sleep SWA topography we calculated a ratio of SWA between females and males for each electrode (figure 1b). A visual inspection of this contrast revealed higher SWA over bilateral temporal regions in females compared to males, while males tended to have higher SWA over central and frontal regions.

For further analysis we employed a cluster analysis (see Methods for details). We found a significant increase of SWA in females compared to males in the right temporal region (R2; p=0.008) and a trend in the left temporal region (R1; p=0.052) (figure 1c). Males, on the other hand, exhibited significantly higher SWA in the right frontal region (R3; p=0.048) compared to females.

For an anatomical localization of the three regions, the corresponding electrodes were co-registered with individual T1-weighted magnetic resonance images (for details see Methods). Orthogonal projection of the electrodes underlying the cluster onto the cortex showed that increased SWA in females was located over Brodmann Area (BA) 22, BA 37, BA 39, BA 42 and BA 47 (figure 1c). All of them are involved in the processing of language. Co-registration of the cluster where males exhibit higher SWA than females revealed localization over BA 9 and BA 10.



	Power difference (SWA %)	t	p	Cluster-size	Localization
R1	15% (f>m)	2.1	0.052	2	BA 22, BA 47
R2	11% (f>m)	3.0	0.008	5	BA 22, BA 37, BA 39, BA 42
R3	14% (m>f)	2.1	0.048	4	BA 9, BA 10

Figure 1: *Topographical distribution of SWA during the first 60 minutes of NREM sleep in females and males a. Averaged power map for females (pink, n=11) and males (blue, n=11). c. Differences of the topographical distribution of SWA between females and males (females/males). Regions colour coded in pink indicated higher SWA in females compared to males. Regions colour coded in blue indicate higher SWA in males compared to females. c. t-map of the corresponding unpaired t-tests for the contrast between females and males. Only electrodes with a probability below 20% (t=1.33) are depicted. Three clusters (R1-R3), each consisting of at least 2 electrodes, were defined. For each cluster the average SWA difference, t-values and p-values of the unpaired t-test comparing females and males, the number of electrodes for each cluster and its functional localization is shown in the table below.*

Because the age range of our sample concerns a period of large developmental changes and of the maturation of diverse skills, we

asked if the observed sex differences are stronger at a certain age. Multivariate ANOVA with the clusters as dependent variable, sex as constant term and age as covariate revealed no significant effect of age (R1, p=0.08; R2, p=0.7 and R3, p=0.8).

Discussion

In this study we used an explorative approach to answer the question if the proposed usefulness of sleep slow wave topography as a marker of cortical maturation is able to uncover sex differences in adolescents. In our sample of children and adolescents we found that females exhibited higher SWA in language associated areas of the left and the right hemisphere compared to age-matched males, while males showed increased SWA over the right prefrontal cortex, a region associated with spatial abilities.

There is growing evidence for a relationship between SWA and synaptic plasticity. Experimental studies, which focused on the effect of learning on SWA, reported local increases of SWA over brain regions where synaptic changes due to learning took place (Huber et al., 2004; Huber et al., 2006; Landsness et al., 2009; Maatta et al., 2010). Synaptic plasticity (i.e. a change in strength and/or number of synapses) not only occurs due to short-term learning, but also in the course of brain maturation. It was recently shown that during development SWA is not equally distributed across the scalp, but exhibits local maxima that are characteristic for a certain age (Kurth et al., 2010a). More specifically, the location of maximal SWA parallels the time course of cortical maturation along the posteroanterior axis (Shaw et al., 2008). Thus, the topography of SWA may reflect synaptic plasticity during development. In an animal model Vyazovskiy et al. provided a mechanistic explanation for the parallel time course of synaptic plasticity and SWA by showing that increased synaptic strength allows for faster synchronization of cortical activity across neurons, which results in larger-amplitude slow waves as observed in scalp EEG recordings (Vyazovskiy et al., 2009). On this background and in line with a recently proposed thalamocortical computer model (Esser et al., 2007) the finding of enhanced SWA in girls compared to boys may indicate greater

synaptic strength of neurons involved in the generation of sleep slow waves in language regions of girls. Such increased synaptic strength may be due to greater density or greater efficacy of cortical synapses or both. In fact, behavioural and anatomical studies support this assumption. Histological analysis showed greater neuronal density (Witelson et al., 1995) and MRI studies proportionally larger grey matter volumes (Harasty et al., 1997) in language regions in adult women, whereas no differences were found in overall cortical thickness (Witelson et al., 1995) or total brain size (Luders et al., 2009). In our sample we have preliminary evidence pointing into the same direction: Girls exhibited larger cortical thicknesses in both language areas (R1: females (mean ± SE): 3.0mm ± 0.2, males: 2.8mm ± 0.3; $p<0.05$; R2: females: 3.2mm ± 0.2, males: 3.0mm ± 0.3, $p<0.05$). No significant difference was found for the frontal area. Recent studies also reported differences in language processing between girls and boys, like increased hemodynamic response or broader involvement of areas participating in the processing of language tasks in girls (Burman et al., 2008). Interestingly, larger activation of language areas was correlated with better language skills and task performance. A reason for the largest sex differences in SWA over the right hemisphere may be the weaker left lateralization of speech functions in females than in men. In fact, left hemisphere injury is less likely to cause aphasia in women than men (Kimura, 2000), which is in line with fMRI studies showing that females exhibit stronger bilateral activation during language tasks, while males seem to rely more on the left hemisphere (Clements et al., 2006; Burman et al., 2008). While females showed increased SWA in areas that are functionally well-defined, in males we found SWA to be enhanced over Brodmann areas 9 and 10, two areas which cover major parts of the prefrontal cortex. The prefrontal cortex is involved in a variety of functions with spatial navigation abilities among others (e.g. (Moffat et al., 2007; Ciaramelli, 2008; Wolbers and Hegarty, 2010), a skill for which an advantage in favour of males has been reported (Kimura, 2000; Wolbers and Hegarty, 2010). Moffat et al. found that better spatial navigation skills were related to larger grey matter volume in the prefrontal cortex (Moffat et al., 2007) which could account for the higher SWA in these regions. However

as the literature is not consistent, this finding should be regarded with caution.

In conclusion, there is growing evidence for a close relationship between sleep SWA and plastic changes. The sensitivity of hd EEG recordings during sleep to uncover sex differences during development reinforces SWA topography as a useful tool to uncover plastic changes. Although hd EEG has a limited spatial resolution compared to magnet resonance imaging (MRI) techniques it may offer some important advantages: it is independent of body position (no immobilisation is needed), is not affected by motivation or vigilance and thus provides a direct and undisturbed assessment of brain activity across the scalp. These advantages may be of particular interest for studies in children and clinical populations.

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Topography of sleep slow wave activity in children with attention-deficit/hyperactivity disorder

Maya Ringli[1], Soraya Souissi[1], Salomé Kurth[1], Daniel Brandeis[2,3,4], Oskar G. Jenni[1,4,6] and Reto Huber[1,4,5,6]

1) Child Development Center, University Children's Hospital Zurich, Zurich, SWITZERLAND

2) Department of Child and Adolescent Psychiatry, University of Zurich, Zurich, SWITZERLAND

3) Department of Child and Adolescent Psychiatry, Central Institute of Mental Health, Mannheim, GERMANY

4) Center for Integrative Human Physiology, University of Zurich, Zurich, SWITZERLAND

5) Neuroscience Center Zurich, University of Zurich, Zurich, SWITZERLAND

6) Children's Research Center, University Children's Hospital Zurich, Zurich, SWITZERLAND

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Abstract

Sleep slow wave activity (SWA, EEG power between 1 and 4.5 Hz) is a major characteristic of non-rapid eye movement (NREM) sleep, which seems to be critically involved in cortical plasticity. Studies using high-density electroencephalography (hd-EEG) showed that the topographical distribution of SWA mirrors cortical maturation, expressing a local maximum that is characteristic for a certain age range. We compared the sleep EEG of children with attention-deficit/hyperactivity disorder (ADHD) with healthy controls to explore differences in sleep SWA. All-night hd-EEG recordings (128 electrodes) were performed in a group of nine children diagnosed with ADHD and nine age- and sex-matched healthy controls. SWA topography was calculated and contrasted between the groups. We found a local increase of SWA in a cluster of six electrodes over central regions in children with ADHD compared to control children (+17% ± 6% SE, $p<0.01$). This group difference was specific for the SWA range and stable across the night. Children with ADHD showed a less mature topographical SWA distribution in comparison to healthy children of the same age and sex. This neuromaturational delay in ADHD is in accordance with neuroimaging and behavioural studies. Thus, our study supports the use of sleep SWA topography as a reliable imaging tool for the study cortical plasticity.

Introduction

Recent evidence suggests that sleep is directly involved in cortical plasticity (Sejnowski and Destexhe, 2000; Steriade and Timofeev, 2003; Born et al., 2006; Tononi and Cirelli, 2006). In particular, slow waves during non-rapid eye movement (NREM) sleep, an electrophysiological marker of sleep depth (Blake and Gerard, 1937), seem to play a key role in synaptic plasticity. The activity of these slow waves (slow wave activity (SWA); EEG power between 1 and 4.5 Hz) is homeostatically regulated, increasing after wakefulness and returning to baseline level during sleep (Borbély and Achermann, 2005). Numerous studies have shown a local regulation of SWA. On the one hand, local increases of SWA were found after intensive use (Kattler et al., 1994; Finelli et al., 2001b) or after learning a specific task (Huber et al., 2004; Landsness et al., 2009). On the other hand, local differences in SWA have also been shown to reflect maturational changes during development, such that SWA was maximal over brain regions maturing at that time (Kurth et al., 2010a). Thus, sleep slow waves seem to be involved in short-term plastic changes due to daytime activity and in long-term plastic changes during development. The relationship of slow waves to daytime activity and to development, both could be of special interest in the context of attention-deficit/hyperactivity disorder (ADHD). ADHD is the most common neurobehavioural disorder during childhood and was shown to affect about 3-12% of school-age children (Faraone et al., 2003). According to the Diagnostic and Statistical Manual of Mental Disorders fourth edition (DSM-IV, American Psychiatric Association 1994) ADHD is characterized by developmentally inappropriate symptoms of inattention and/or impulsivity and hyperactivity. Although no general consensus exists about the genesis of ADHD so far, there is growing evidence from behavioural (Drechsler et al., 2005; Doehnert et al., 2011), as well as neuroimaging studies (Shaw et al., 2006a; Shaw et al., 2007; Shaw et al., 2011) for a maturational delay in neurodevelopment of ADHD children. In fact, children diagnosed with ADHD may show both, a delay in normal cortical maturation, as well as an intensive use of the motor cortex due to motor hyperactivity. So far, the vast majority of

studies investigating brain functions in ADHD subjects were performed during wakefulness, when subjects are conscious and active (Durston, 2003; Bush et al., 2005). Only few studies have investigated sleep in ADHD. These studies, however, remain inconclusive for the understanding of the function of sleep in ADHD. Moreover, studies investigating basic electrophysiological sleep mechanisms are still largely missing.

Here we used high-density sleep EEG, a tool that enables the mapping of EEG power with high spatial resolution, in a sample of children diagnosed with ADHD with the goal to address regional maturational and/or use dependent differences in SWA topography.

Methods and materials

Participants

Nine subjects (8 males, 1 female), mean age 11.9 years (range: 9.7 – 13.4 years) meeting the criteria for ADHD as defined by the DSM-IV (American Psychiatric Association 1994) were recruited from the Department for Child and Adolescent Psychiatry at the University of Zurich. The diagnosis was based on a semi-structured interview (Parental Account of ADHD Symptoms (PACS) (Taylor et al., 1996) and the Conners Teachers' Rating Scales-Revised (CTRS-R:L) (Conners et al., 1998) (for details see (Muller et al., 2011)). All children met the ADHD combined type criteria. 7 children were free of use of psychoactive agents or other medication. Two children were treated with methylphenidate with daily doses of 15mg and 20mg (2 doses of 10mg), respectively. Medication, respectively the second dose, was not given at the day of measurement. Subjects underwent a telephone and questionnaire screening to exclude co-morbid psychopathologies, chronic diseases and sleep disorders (including sleep disordered breathing and periodic limb movements during sleep).

Healthy control subjects were selected from an ongoing study in our lab (for details see (Kurth, et al. 2010). Controls were sex- and age-matched (mean age 11.6, range: 9.6 – 14.2 years). None of the control children reported the use of psychoactive agents or other medications. They confirmed the absence of any inattentive and

hyperactive-impulsive symptoms. Criteria for ADHD as defined by DSM-IV were not met. Subjects underwent a telephone and questionnaire screening to exclude personal and family history of psychopathology, chronic diseases and sleep disorders (including sleep disordered breathing and periodic limb movements during sleep).

All subjects were right-handed and non-smokers. Written informed consent was obtained from the parents and the children after careful explanation of the study methods and aims. The procedures were approved by the local ethics committee, and the study was performed according to the Declaration of Helsinki. Six month prior to the study no participant travelled across more than one time zone. One week prior to the study, all participants were instructed to maintain regular sleep-wake schedules according to their habitual bedtimes and to keep their caffeine consumption on restricted levels (i.e., less than one serving per day). Compliance was monitored with self-reported sleep logs and wrist motor actigraphy. 24 hours before and during the course of the study, they were asked to refrain from alcohol and any medication and to avoid naps.

Recording and preprocessing of EEG data

All EEG data were collected in the sleep laboratory of the University Children's Hospital Zurich with a hd-EEG device (Electrical Geodesic Sensor Net for long-term monitoring, 128 channels). The nets were adjusted to the vertex, and the cap electrodes were filled with gel electrolyte. Impedances were measured at the beginning of the recording and kept below 50kΩ. The sleep episode of each subject was scheduled according to habitual bedtimes. To allow for regular school participation participants were awakened in the morning, resulting in variable rise times.

EEG recordings were sampled at 500Hz (0.01 - 200Hz) and referenced to the vertex (Cz). The data was next band-pass filtered between 0.5 and 50 Hz and downsampled to 128Hz. Artefacts were rejected on a 20s basis after visual inspection and if power exceeded a threshold based on a mean power value in the 0.75-4.5 and 20-30Hz bands (Huber, et al. 2000). After exclusion of EEG channels of

insufficient quality (on average, two channels per subjects) the data were re-referenced to average reference.

After visually scoring for sleep stages (20s epochs, American Academy of Sleep Medicine standard criteria (Iber et al., 2007)) non-rapid eye movement (NREM) sleep episodes were defined according to standard criteria (Rechtschaffen and Kales, 1968; Feinberg and Floyd, 1979) and adapted because of frequently occurring “skipped” rapid eye movement (REM) sleep after the first NREM sleep episode (for details see (Kurth et al., 2010a)).

EEG Power analysis and statistics

For a quantitative analysis of the sleep EEG, spectral analysis of consecutive 20-s epochs (FFT routine, Hanning window, averages of five 4-s epochs) was performed for all channels. SWA was calculated as mean power in the range of 1-4.5 Hz during the first 60 minutes of NREM sleep stage 2 and 3. EEG power for each electrode was normalized to the average of all electrodes. To assess significant topographical differences in SWA between the two groups, we applied statistical nonparametric mapping (SnPM) using a suprathreshold cluster analysis for multiple comparisons (Nichols and Holmes, 2002; Huber et al., 2004; Ferrarelli et al., 2007).

Anatomical localization of electrodes was verified in eight subjects of the control group using magnetic resonance imaging (MRI) and the positioning software SofTaxic Optic (EMS Inc). Electrodes were digitized and co-registered with the subject’s MRI (for details see (Kurth et al., 2010a)).

Table 1. *Sleep variables (mean ± SE) of all subjects, presented separately for each group. Sleep latency, latency to the first occurrence of stage 2 sleep; Rem latency, latency to the first occurrence of stage Rem sleep; Wake after sleep onset, expressed in minutes; Sleep stages are expressed in minutes and as a percentage of total sleep time; REM sleep, rapid eye movement; Total sleep time, total duration of time spent in sleep stages 1-3 and REM; Total time in bed, time when recording begun until it was stopped including wakefulness; Sleep efficiency, Total sleep time expressed as a percentage of time in bed. Values that are marked with a star * are significant different between groups. All variables were compared between groups, using unpaired t-tests. Only the comparison of sleep stage 1 in minutes and percentage reached significance ($p<0.05$).*

Sleep variables	ADHD mean (± SE)	Controls mean (± SE)
Sleep latency (min)	18.6 (± 3.5)	24.9 (± 5.1)
REM latency (min)	145.6 (± 17.8)	165.2 (± 21.7)
Wake after sleep onset	39.9 (± 11.2)	44.9 (± 12.0)
Sleep stage 1 (min)	21.2 (± 4.8)*	34.4 (± 5.6)*
Sleep stage 1 (%)	4.5 (± 1.0)*	7.4 (± 1.1)*
Sleep stage 2 (min)	225.8 (± 7.1)	222.9 (± 16.9)
Sleep stage 2 (%)	47.2 (± 1.8)	48.3 (± 2.5)
Sleep stage 3 (min)	118.6 (± 10.5)	106.9 (± 9.1)
Sleep stage 3 (%)	24.8 (± 2.2)	23.5 (± 2.5)
Rem (min)	114.6 (± 13.7)	96.9 (± 10.4)
Rem (%)	23.6 (± 2.3)	20.8 (± 1.6)
Total sleep time (min)	480.3 (± 15.5)	461.0 (± 21.2)
Total time in bed (min)	539.3 (± 9.4)	526.8 (± 20.5)
Sleep efficiency (%)	89.0 (± 1.8)	87.6 (± 2.8)

Results

General sleep quality was assessed on visually scored sleep variables (Table 1). Sleep quality was good in both groups (i.e. showing high sleep efficiency). All sleep stage measures were comparable between the two groups and no significant differences

were found, except for the amount of sleep stage 1, with the control group exhibiting more stage 1.

To investigate the topography of sleep SWA in children with ADHD we calculated power maps for each group (Figure 1A). The distribution of SWA showed regional differences with local maxima and a symmetrical distribution (Figure 1B). When contrasting the maps, we found local differences in SWA over central and frontal areas (Figure 1C). Compared to age- and sex-matched controls, ADHD children exhibited 17% (± 6% SE, $p<0.01$) more SWA in a cluster of six central electrodes (SnPM, see Methods and Materials for details; Figure 1C).

For an anatomical localization of the central cluster we co-registered the electrodes to individual T1-weighted magnetic resonance images (for details see Methods and Materials) in eight healthy controls. Orthogonal projection of the electrodes onto the cortex localized five of the electrodes to the frontal cortex (Brodmann area 6) and one to the primary somatosensory cortex (Brodmann area 3) (Table 2).

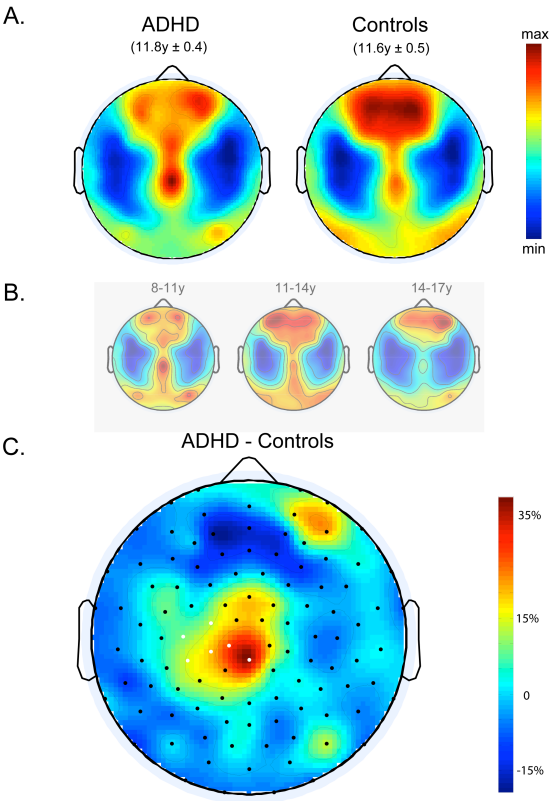

Figure 1. *Topographical distribution of SWA (1-4.5 Hz), based on the first hour of NREM sleep stages 2 and 3. A. Maps were normalized across all electrodes for each individual and then averaged for each group. Values are color coded (maxima in red, minima in blue) and plotted on the planar projection of the hemispheric scalp model. B. Representative topographic distribution of SWA of three age groups illustrating the typical maturational changes (adapted from Kurth et al., 2010). C. Topographical distribution of the difference in SWA between ADHD and controls (ADHD minus Controls). Values are color coded (group differences in percentage). SWA was increased by 17% (± 6% SE) at a central cluster of six significant electrodes, indicated as white dots (p<0.01, statistical nonparametric mapping, supra-threshold cluster test controlling for multiple comparisons (Nichols and Holmes, 2002)).*

Electrode number	Talairach coordinates (mean ± SE)			Anatomical localization [lobe, gyrus, Brodmann area (BA)]	Range (mm)
	x	y	z		
30	-29 (3)	14 (3)	56 (2)	Frontal lobe, middle frontal gyrus, BA 6	0
31	-13 (3)	-1 (5)	67 (1)	Frontal lobe, superior frontal gyrus, BA 6	3
36	-43 (2)	5 (4)	48 (2)	Frontal lobe, middle frontal gyrus, BA 6	1
37	-29 (3)	-6 (4)	62 (2)	Frontal lobe, middle frontal gyrus, BA 6	0
42	-45 (3)	-16 (3)	53 (2)	Parietal lobe, postcentral gyrus, BA 3	0
55	-2 (3)	-11 (3)	71 (1)	Frontal lobe, medial frontal gyrus, BA 6	4

Table 2. *Anatomical localization of the six electrodes that belong to the cluster expressing the local increase of SWA between the ADHD and the control group. Electrodes were digitized and co-registered with the subject's magnetic images. Average ± SE Talairach coordinates are indicated for 8 subjects. The corresponding lobe, gyrus, and Brodmann area were detected using the Talairach Client (Lancaster et al., 1997; Lancaster et al., 2000). The range (in millimeters) refers to the distance of the nearest gray matter area with respect to the coordinates.*

We then investigated the stability of this group difference in the course of the night. Contrasting the SWA map for the first five sleep cycles revealed that the local increase of SWA in ADHD was stable across the night (data not shown). We then asked if this group difference was specific for the SWA frequency range. For this reason we compared the average frequency spectrum within the cluster of central electrodes between groups which showed that the group difference was specific for low frequencies (1-3Hz) (Figure 2).

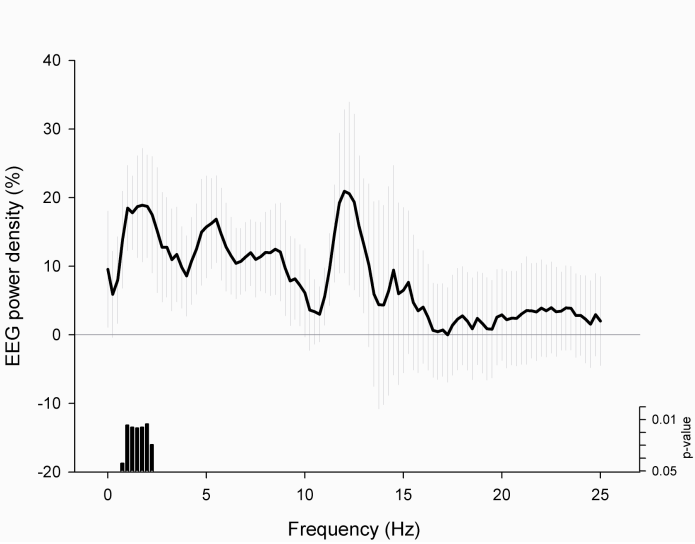

Figure 2. *EEG power density spectrum for the first 60 minutes of NREM sleep. Values represent the percentage change between the ADHD group with respect to the control group (mean ± SE for 0.25-Hz bins, n= 9 (per group)). Black curve: Average power change across ADHD subjects of all six electrodes in the cluster. Bottom bars indicate frequency bins for which power in the ADHD group differed significantly from the control group (unpaired t-test).*

Discussion

Our study shows that the topographical distribution of sleep SWA in ADHD children resembles a general pattern known from healthy children (Kurth et al., 2010a). However, children with ADHD showed

an increased power over central areas when compared to age- and sex-matched healthy controls.

With the exception of a small increase in the amount of stage 1 sleep, we did not find any differences in measures of sleep architecture between ADHD and control children. Both groups slept equally well with a similar initiation of sleep (sleep latency), good sleep efficiency and a comparable time course of SWA across the night (data not shown). These results are in line with earlier studies reporting no differences in NREM sleep between ADHD and controls (Busby et al., 1981; Greenhill et al., 1983; Konofal et al., 2001; Kirov et al., 2004).

The strength of our study is the use of hd-EEG, instead of conventional EEG, with higher spatial resolution. Such hd-EEG recordings during sleep in children and adolescents showed age-related regional differences in the SWA band, but not in other frequency ranges (Kurth et al., 2010a). We found that children with ADHD exhibited a typical topographical distribution of SWA with a clear local maximum, sustained symmetry and bilateral temporal power minima. However, when the maps were contrasted between the groups, children with ADHD showed significantly more SWA over central regions (Figure 1). This increase was specific for the slow frequency range (Figure 2) and stable across the night (data not shown). We discuss two possible explanations accounting for this topographical variation in ADHD children.

The topography of SWA is characterized by local maxima which show an age-dependent shift from occipital areas during early childhood, central areas in late childhood to frontal areas in late adolescence (Kurth et al., 2010a). Imaging studies are in line with this observation, showing that cortical maturation also follows a posterior–anterior time course, with lower-order primary areas maturing first, followed by higher-order association areas (Gogtay et al., 2004; Sowell et al., 2004). Thus, the local difference in SWA found in children with ADHD could be interpreted as a maturational delay. This idea of a neuromaturational delay is not new. Because core symptoms of ADHD do not seem to be pathological per se, but rather occur in an untypical way with reference to the child's age, the idea of a maturational delay as the underlying cause of the disorder has been

proposed by several groups (Kinsbourne, 1973; Drechsler et al., 2005; Shaw et al., 2007; Gustafsson et al., 2010; Shaw et al., 2010; Doehnert et al., 2011). The idea is supported by the fact that the symptoms of ADHD tend to improve with age (Faraone et al., 2003; Faraone et al., 2006). In addition, behavioural data showed that the degree of cognitive deficits observed in children with ADHD vary as a function of their developmental order, such that the deficits are most pronounced during the time when a particular skill is developed in healthy children (Drechsler et al., 2005). Recent evidence also comes from imaging studies, showing that in children with ADHD the onset of grey matter maturation is delayed by around 3 years compared to healthy controls while the developmental trajectory itself is not deviant (Shaw et al., 2007). Moreover, remission of ADHD symptoms was related to cortical normalization, implying a 'maturational catch-up' that is accompanied by an improvement of symptomatology (Shaw et al., 2006b). Remarkably, cortical maturation is related to core symptoms of ADHD not only in children diagnosed with ADHD, but also in healthy children expressing the behaviour on normal levels (Shaw et al., 2011). Since slow waves originate from synchronized activity of cortical neurons (Steriade et al., 1993; Steriade et al., 2001; Timofeev et al., 2001; Vyazovskiy et al., 2009) a close relationship between SWA and cortical plasticity can be inferred (Tononi and Cirelli, 2006). More specifically, in an animal model it was shown that increased synaptic strength allows for faster synchronization of cortical activity across neurons, which results in larger-amplitude slow waves as observed in scalp EEG recordings (Vyazovskiy et al., 2009). On this background it is not surprising that cortical changes are mirrored in the activity of the sleep slow waves. Hence, we would expect highest values of SWA over those regions where major maturational processes are taking place (Gogtay et al., 2004; Luna and Sweeney, 2004; Sowell et al., 2004). In our ADHD children, the local maximum of SWA along the posterior-anterior axis was located behind that of the control subjects. Thus, this result supports a neuromaturational delay in children with ADHD. However, this finding needs to be confirmed by longitudinal studies which allow the investigation of SWA maturation on the intraindividual level, which is needed to draw reliable conclusions about developmental properties.

Still other reasons may account for the difference in SWA topography. For instance, it was shown that SWA can locally increase as a result of a more intense use during preceding wakefulness (Kattler et al., 1994; Finelli et al., 2001b). Both sleep deprivation (Finelli et al., 2001a) and extensive stimulation of the somatosensory cortex (Kattler et al., 1994) produced an increase of SWA during consecutive sleep compared to a baseline night, over the region that was stronger activated during the day. In our study, the local increase of sleep SWA in children with ADHD was found over the central cortex, covering medial parts of the primary and secondary motor cortex. Based on the close relationship between daytime activity and sleep SWA, the local increase of SWA found in ADHD children could also be use-dependent, reflecting motor hyperactivity, one of the core symptoms of ADHD.

Although both explanations seem to be plausible and are even likely to be linked, there are two additional observations favouring the maturational hypothesis: First, if the local increase of SWA was use-dependent, we would expect a reduction of the group difference in the course of the night, because of the homeostatic regulation of SWA (Borbély and Achermann, 2005). The homeostatic regulation of SWA is exemplified by sleep deprivation studies. Thus, sleep deprivation results in higher initial SWA which dissipates in the course of sleep converging to baseline levels at the end of the night (Borbély and Achermann, 2005). In our study, however, the local SWA difference remained stable across the night. Secondly, we tested if the local increase of SWA could just be the result of increased daytime activity. We therefore analyzed daytime activity, which was assessed by continued monitoring of motor activity with wrist actigraphy. In our control subjects average daytime activity was positively correlated to SWA over the contralateral motor cortex ($R=0.7$, $p<0.05$, mean SWA in a cluster of 3 electrodes). However, no such relationship was found in the ADHD group. This finding weakens the idea that the local increase of SWA in our ADHD children could be the result of pure motor hyperactivity.

In conclusion, this study provides evidence for a neuromaturational delay in children diagnosed with ADHD, using a novel marker of

cortical maturation. In comparison to conventional techniques, the use of SWA topography as an imaging tool provides several advantages. First, SWA topography directly reflects variations in the underlying spontaneous neuronal activity (Steriade et al., 2001; Timofeev et al., 2001; Vyazovskiy et al., 2009). Such neuronal activity may be a more direct predictor of functional differences than brain anatomy. Second, the assessment of changes in neural activity during sleep minimizes possible confounding factors related to waking activities, including changes in the level of attention and distractibility, and issues of motivation or cognitive capacity. The reduction of such confounding factors might be especially relevant for studies investigating changes in brain activity in children and patients with cognitive and/or behavioural impairments. The robustness of this measure is also illustrated by its high reproducibility between two nights (Finelli et al., 2001b; Huber et al., 2004). Third, in clinical populations, and particularly in children, the amount of data that can be collected is often very limited (i.e., covering only a few snapshots (minutes) of brain activity). Data collection during sleep, however, allows the data collection of hours of brain activity resulting in a stable assessment. Moreover, the well known changes in the sleep EEG across the night offer the possibility to assess changes in brain activity under different conditions during the same night, which has provided insight into pathological processes in the past (Bölsterli et al., 2011). Thus, we suggest that – although most researchers focus on wakefulness to understand ADHD – sleep may be a rich source of information to help understand healthy and disturbed processes of brain function and development.

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Concluding Remarks

GENERAL DISCUSSION

The studies enclosed in the present thesis support a close relationship between sleep SWA and cortical plasticity during development. The major changes in slow wave sleep during development were presented in a general overview and related to cortical maturation and behaviour (Chapter 2) In addition, we proposed a model of how SWA may contribute to the regulation of synaptic density during childhood, adolescence and adulthood, resulting in the typical inverted U-shaped time course. We then demonstrated that the decrease of SWA during adolescence is correlated to the decline in grey matter volume (Chapter 3). For the first time the SWA topography was mapped across the first two decades of life and was shown to parallel the time course of cortical maturation during childhood and adolescence (Chapter 4). Thus, we established SWA as a marker of cortical maturation, which mirrors age-related changes in brain activity in the course of development. Additionally, the SWA topography was successfully applied as a tool to uncover age-independent functional differences between males and females (Chapter 5), as well as delayed maturation as a possible factor of disturbed behaviour in children with ADHD (Chapter 6)

In our first study (Chapter 3) we have investigated the relationship between changes in grey matter and alterations in EEG power, especially in the low frequency range. The decreases in SWA and grey matter were highly correlated, as previously proposed (Feinberg, 1982), but never investigated before. Furthermore, the relationship between SWA and grey matter was most prominent in areas maturing during adolescence and strongest for the SWA frequency range. An important additional finding was that this correlation also hold true after correcting for age. This means that SWA explains more variability in cortical maturation than age. Larger grey matter volumes may at least in part arise from higher synaptic and cell density during childhood (Brewer et al., 2009), which is also confirmed by the simultaneously higher energy consumption (Chugani, 1987). Therefore, SWA represents a good marker for structural changes in neuronal networks reflecting cortical maturation during adolescence.

The close relationship between the timing of cortical maturation and SWA was also shown by the investigation of regional differences of SWA (Chapter 4). The use of high-density EEG enabled the mapping of the distribution of SWA across the scalp with high spatial resolution. Since it was shown that lower-order primary areas mature early and higher-order association areas mature rather late during development (Gogtay et al., 2004; Sowell et al., 2004; Shaw et al., 2008), we expected regional differences in SWA according to the timing of maturation. Indeed, the analysis of SWA topography uncovered relative power differences in SWA, with local maxima over the brain region maturing at that time. It was therefore concluded that SWA may be a marker of cortical maturation and its topography may serve as a tool to map the current state of cortical maturation. Indeed we successfully detected local differences in brain maturation between children with ADHD and healthy controls by investigating the SWA topography (Ringli et al., submitted-b).

However, cortical maturation does not seem to be reflected only in local aspects, as is shown in the regional differences described above, but also in global aspects, as we have discussed in our review (Chapter 2). In this work we have highlighted the major changes in sleep slow waves during development and linked them to cortical

maturation and behaviour. One important observation is that both synaptic density and slow-wave activity increase during childhood and decline in the course of adolescence, reaching overall stable levels during adulthood (Huttenlocher, 1979; Feinberg, 1982). We then addressed the question whether SWA is merely reflecting synaptic changes or if it plays an active role in brain maturation. We proposed a possible mechanism by which sleep SWA may contribute to cortical maturation. The model suggests that while there is a balance between synaptic strengthening and synaptic downscaling in adults, the balance of strengthening/formation and weakening/elimination is tilted during development. Support for this hypothesis comes from a recent animal study showing that indeed there is a net loss during adolescence, demonstrating higher spine loss during sleep than spine gain during wakefulness, whereas spine turnover was balanced in adults (Maret et al., 2011). Additionally, recent human work provides evidence for an active role of SWA. In this study, the SWA topography of normally developing children was related to the performance level of different skills in the same subjects (Kurth et al., submitted). Although both measures showed a similar time course, they were shifted in timing, such that the maturation of a particular brain region, as reflected by SWA, preceded the maturation of the corresponding skill by approximately three years. Moreover, also grey matter followed the same developmental pattern but seemed to be the last to mature. Thus SWA possibly triggers the remodelling of brain circuits through downscaling by first enhancing synaptic density during childhood and later by reducing the number synapses (pruning) during adolescence, both resulting in improved performance. The timing of grey matter maturation may be explained by the fact that changes in grey matter as measured by MRI not only reflect changes in the number of synapses, but are also influenced by changes in hydrophobic lipids and iron content, or the age-related increase in myelination (Steen et al., 1997; Paus et al., 2001).

Finally SWA topography was applied as a tool to uncover local differences between groups. The study of sex differences revealed increased SWA over language areas and decreased SWA in a frontal area associated with spatial processing, in girls compared to boys

(Chapter 5). This finding is in line with reports showing that females outperform males in many language skills (Kimura, 2000; Plante et al., 2006; Burman et al., 2008) while boys excel girls in spatial abilities (Feng et al., 2007; Tzuriel and Egozi, 2010). Based on the observation that SWA reflects synaptic changes (Tononi and Cirelli, 2006), this finding may indicate either stronger activation because of higher use during the day (Kattler et al., 1994; Finelli et al., 2001b) or general differences in the underlying neuronal brain circuits. The latter may arise from learning related remodelling (Huber et al., 2004; Huber et al., 2006; Huber et al., 2007b) or be the result of biological predisposition, as functional sex differences seem to be present already early in life (Huttenlocher et al., 1991; Moore and Johnson, 2008; Quinn and Liben, 2008; Ozcaliskan and Goldin-Meadow, 2010). Similarly, we found topographical differences in SWA in children suffering from ADHD (Chapter 6). Compared to age- and sex-matched healthy controls, children with ADHD exhibited increased SWA over central regions. Since the maturation of SWA topography showed a posterior-to-anterior timecourse (Kurth et al., 2010a) and in accordance with reports of different neurodevelopmental trajectories between ADHD and healthy controls (Shaw et al., 2006b; Shaw et al., 2007) we concluded that the pattern found in ADHD patients reflects a maturational delay in brain development. Alternatively we have discussed the possibility that the increase of SWA may be the result of the predominant hyperactivity in children with ADHD inducing stronger activations of motor areas. However, two findings provide evidence in favour of the maturational delay hypothesis: First, if the local increase of SWA was use-dependent, we would expect that the group difference would diminish in the course of the night, because of the homeostatic regulation of SWA (Borbély and Achermann, 2005). In our study, however, the local SWA difference remained stable across the night. Second, average daytime activity, as measured with wrist-actiwatch, was positively correlated to SWA of the contralateral motor cortex in the control group, but not in the ADHD children. Therefore, it is less likely that the local increase of SWA in our ADHD children may be just the result of pure motor hyperactivity.

In summary, the work discussed above support sleep SWA as a marker of cortical plasticity in the course of anatomical reorganization during brain development, as well as of functional differences between different populations. Thus it suggests the investigation of SWA topography to study normal and pathological mechanisms.

NEW QUESTIONS AND FUTURE RESEARCH

In the preceding section the major findings of the thesis have been summarized and discussed. In particular, the demonstration of SWA topography as a sensitive tool to display local differences in cortical plasticity is promising for future research and clinical application. In the following sections these two aspects are discussed in more detail. First the focus is set on the nature of possible differences in SWA topography by considering maturational versus use-dependent changes. Second, possible implications on diagnosis, treatment and future research in ADHD are presented. Finally, the section closes by highlighting the importance and the potential of this research. The last part provides an outlook of how SWA may be investigated in the future.

Differences in the SWA topography: use-dependent or maturational origin?

In the present thesis two studies have been included which introduced the possibility of mapping anatomical and functional differences between two groups with hd-EEG during sleep. The comparison of boys and girls has revealed differences in SWA over language associated areas and over a region involved in spatial abilities. In addition, the comparison of the SWA topography between healthy children and ADHD patients exhibited a local increase of SWA over the central cortex in the latter group. According to the synaptic homeostasis hypothesis (Tononi and Cirelli, 2006) and based on numerous studies confirming a close relationship between synaptic changes and SWA (Esser et al., 2007; Vyazovskiy et al., 2008; Vyazovskiy et al., 2009b) we have interpreted theses findings as reflecting group differences in the underlying functions.

Up to now, in the literature, differences in SWA topography were clearly assigned to either use-dependent (Kattler et al., 1994; Finelli et al., 2001b) or maturational (Kurth et al., 2010a) origin. The clear distinction was possible, because the studied subjects were either healthy mature males, so differences are most likely the result of the applied experimental manipulation, or they were healthy developing children of different ages, so differences are most probably age-dependent. In contrast, in the two last studies enclosed in this thesis, the subjects were developing children of the same age, which differed in respect to specific functions, due to their sex or a disorder. In this case, how are effects of maturation disentangled from use-dependent influences?

In the preceding section the question was discussed if the local differences between ADHD and healthy children are due to distinct levels of use or rather mirror different maturational trajectories. Although there seems to be more evidence in favour of the maturational hypothesis, still both mechanisms would be plausible origins of the difference. Interestingly, both explanations could also account for the sex differences. There is evidence for an association between cortical maturation and quality of performance: First of all, different trajectories of cortical development were reported between boys and girls (Giedd et al., 1999; Lenroot and Giedd, 2010; Raznahan et al., 2010; Porter et al., 2011). Second, cortical thinning, an indicator of cortical maturation, was related to improvement of language skills (Sowell et al., 2004). From this it could be concluded, that enhanced language skills in girls are due to advanced cortical maturation. Third, behavioural sex differences in language tasks depend on age and task (Burman et al., 2008), which points to developmental changes, because language skills are acquired gradually. Finally, the persistence of theses differences into adulthood (Kimura, 2000; Sowell et al., 2007; Luders et al., 2009; Lenroot and Giedd, 2010) are often quoted as an argument for distinct maturational time courses, in that once the maturation window is closed, also the possibility to catch up to the performance level of the other sex has waned. However, this last observation could also represent support for the use-dependency explanation. There may be just different levels of use of certain skills between both sexes during

development and maintained into adulthood. Indeed, studies have reported changes in performance in language skills (Moura et al., 2009) as well as spatial abilities (Feng et al., 2007; Tzuriel and Egozi, 2010) after having completed a corresponding training. More explicitly, before training, boys outperformed girls at baseline. At follow up however, trained girls performed better than boys of the untrained control group. Nevertheless, they still performed worse than the trained boys (Tzuriel and Egozi, 2010). These findings point to the fact that sex differences may to some extend depend on experience. Still, the initial sex difference at baseline was maintained throughout training. In fact, sex differences are already present in the first years of life (Huttenlocher et al., 1991; Moore and Johnson, 2008; Quinn and Liben, 2008; Ozcaliskan and Goldin-Meadow, 2010). Based on these findings we conclude that it may not merely be a question of which factor, maturation or experience, but rather how both factors contribute to the differences observed in the SWA topography. This aspect, i.e. interplay between maturation (nature) and use-dependency (nurture) is discussed in more detail in the next section.

Experience and timing during brain maturation

Neuronal development is characterized by competing processes of formation and elimination of neuronal branches and synapses and it has been suggested that experience may play a major role in this processes. Early work by Hubel and Wiesel demonstrated that adequate stimulation during a critical period is needed to establish a properly functioning architecture in the visual system and even determines the final outcome without further possibility of correction (Wiesel and Hubel, 1963). Although this may be an extreme example and the brain may be much more plastic in many other brain regions, it demonstrates that experience is vital for normal development. On the other hand, some experience is not necessary, but contributes to functional differences between individuals. The interplay between biological and environmental influences has also been described as “experience-expected” versus “experience-dependent” mechanisms (Galvan, 2010). “Experience-expected” means that a certain activity is expected to occur roughly at the same time point during development in all humans (i.e. visual input begins shortly after birth). On the other hand, “experience-dependent” processes describe

individual opportunities for certain experiences, like the availability of music in a family.

These two forms of experience may also shape the development of a skill as is demonstrated in the example of language acquisition: Auditory stimulation is required (“expected”) for infants to develop language (Kyle, 1980), and basic verbal abilities are acquired starting during the first two years. However, the later refinement of verbal skills, like for example the kind and amount of vocabulary that is obtained is much more “dependent” on the environment. This relationship may also be reflected in the fact that brain regions maturing early (e.g. primary sensory and motor cortex) exhibit greater genetic effects earlier in childhood, while regions maturing later (e.g. frontal cortex) seem to be more heritable during adolescence (Lenroot et al., 2009). This means that the development of each brain region is expected to happen at a certain time, during which the concurrent activity may be of importance.

The effect of experience was also observed on the molecular level. It was shown that the intensity of stimulation induces corresponding changes in the number of synapses: E.g. an increase in synapse number and dendritic branching was observed following enriched environments, while sensory deprivation decreased synapse number (Greenough et al., 1973; Fiala et al., 1978; Knott et al., 2002). Moreover, sensory input influences the formation of synapses through biasing the sites of synaptogenesis (Lendvai et al., 2000). Also, it was observed that neuronal activity modulates the formation rate of new or the stabilization of existing connections (Hua and Smith, 2004). Interestingly, timing seem to be related also to compensatory effects: During adolescence net destabilisation following sensory deprivation was prevented by reducing the elimination rate rather than increasing the formation of spines (Zuo et al., 2005a).

Finally, activity driven plasticity is also found in sleep. Raising cats or mice in complete darkness from birth resulted in a strong reduction of slow wave activity in the primary visual cortex which recovered gradually after re-exposure to light (Miyamoto et al., 2003). In contrast, dark-rearing of mature animals did not affect SWA

(Miyamoto et al., 2003) in line with the finding that also light-deprivation well after the critical period of ocular dominance plasticity has little effect on receptive field properties (Daw, 1995). Furthermore, sleep was shown to increase the effects of monocular deprivation on the visual cortex in kittens whereas preventing the animals from sleep reduced the influence of sensory deprivation on the visual cortex (Frank et al., 2001). Recent evidence suggests that the effect of visual deprivation is regulated by neuronal activity during sleep (Jha et al., 2005). Therefore sleep in early life seems to play an essential role in development.

In summary, timing and experience play an important role in brain development. Moreover these processes also seem to be regulated by sleep. While some experience is contributing to the variability of human individuals, other experience is vital for proper development. Interestingly, also processes thought to be under developmental constraint can be influenced by the manipulation of the environment: Children who received enriched reaching experience developed object exploration faster compared to healthy controls (Needham et al., 2002). Together these findings show the dynamic nature of brain development which may also be the reason for the high vulnerability for the onset of psychopathologies during development, especially in the phase of adolescence (Paus et al., 2008).

Going back to the initial question about the origin of differences between sexes or between healthy children and ADHD patients, we have to conclude that the nature of the observed difference is maybe harder to understand than previously thought. The finding, that neurodevelopment is delayed in children with ADHD does not tell us what causes this delay. As outlined earlier, enriched experience may speed up development (Needham et al., 2002). The opposite may occur in ADHD: It could be hypothesized that due to impaired attention the interaction with the environment may be disturbed. As enriched experience fastens maturation, limited interaction with the environment may slow down development. Similarly in sex differences: Although the developmental trajectories may not be the same in boys and girls and cause the observed differences in brain anatomy and behaviour, it could also be that only the susceptibility to

language or spatial stimuli is biologically determined. This would then bias the use of one or the other skill and thus result in activity dependent differences. We cannot answer these questions on the basis of our data set. Longitudinal studies, starting early in life and combining sleep recordings with brain imaging and behavioural data may help understand the interaction between activity- and maturation-driven processes. Despite these limitations, still the findings have an impact on current interventions in ADHD.

Impact on interventions in ADHD

Currently, the prescription of psychostimulants is the intervention of choice to treat ADHD and has tremendously increased in the last decades (Zito et al., 2000; Comer et al., 2010). Analyses on the use of psychotropic medication in the 90's report a prevalence of 12.3% for stimulants in preschool children, whereof 90% were represented by methylphenidate (Zito et al., 2000). Besides the diversely discussed benefit and long term outcomes of pharmacological treatment in children, the issue is also of relevance in light of our finding of a neuromaturational delay in children with ADHD. Since the SWA topography of children with ADHD showed a less mature pattern compared to healthy subjects, the question arises if and how pharmacological interventions, especially the treatment with methylphenidate, influence brain development. Moreover, because there is growing evidence for a link between SWA proper learning (Huber et al., 2004; Marshall et al., 2006; Aeschbach et al., 2008; Landsness et al., 2009; Bölsterli et al., 2011), the effect of drug treatment on the generation of slow waves and the synaptic downscaling are important to investigate, since learning processes seem to be disturbed in children with ADHD (Spencer et al., 2007). Last, the question arises about the impact of our finding on non-pharmacological therapeutic approaches. In the following these aspects are discussed in more detail.

Effects of medical treatment on brain maturation

In humans, psychostimulants were shown to suppress growths rates in height and weight in children (Faraone et al., 2008). Together with the observation of delayed grey matter maturation in untreated

children with ADHD (Shaw et al., 2006a; Shaw et al., 2007) it may be asked, if psychostimulants also suppress cortical development in children with ADHD. Recently a study addressed this question, by investigating the rate of change in cortical thickness in three groups: ADHD patients treated with psychostimulants, ADHD without medical treatment and healthy controls (Shaw et al., 2009). Surprisingly, no slowing of overall cortical maturation was found in adolescents with ADHD taking psychostimulants. In contrast, more rapid regional decreases of cortical thickness were found in non-treated adolescents with ADHD, compared to both healthy controls as well as ADHD patients taking medication. This finding could be interpreted as experience-driven plasticity: Since psychostimulants were shown to benefit numerous cognitive processes (Pietrzak et al., 2006), which also seem to increase neuronal activity (Rubia et al., 2011), it could be concluded that medication has an indirect positive influence on brain development, because better focussing may lead to a better interaction with the environment and therefore may result in more intense and more specific brain stimulation. Evidence for this hypothesis comes from a training study, which reported that performance improvement after attention training was also followed by changes in the EEG towards a more mature pattern (Rueda et al., 2005).

Effects of medical treatment on SWA and downscaling

Effects on slow wave sleep have been demonstrated for many substances. For example in adults it was shown that low doses of caffeine, a psychostimulant, lead to prolongation of sleep latency and reduced SWA in the following night (Landolt et al., 1995). Similarly in animals, caffeine had a suppressing effect on slow waves in juvenile rats (Olini et al., unpublished data). Moreover, caffeine seemed to alter normal brain development. Also, many benzodiazepines were shown to inhibit SWA (Aeschbach et al., 1996), and impair memory processes (Mejo, 1992). Unfortunately, the effect of methylphenidate of slow wave activity has not been investigated so far, whereas the influence of methylphenidate on sleep in general is diversely discussed. While some studies reported an increase of sleep stage 2 and overall improvement of sleep complaints (Kim et al., 2010), others have shown no changes in sleep architecture but a general

reduction of sleep quantity (Galland et al., 2010). The effect of methylphenidate depends on the inhibition of monoaminergic reuptake transporters of the presynapse (Prince, 2008; Wilens, 2008). Substances acting as monoaminergic inhibitors were shown to increase wakefulness and decrease slow wave sleep (Nishino et al., 1998). However, studies investigating the effects of methylphenidate on the generation of slow waves and the homeostatic regulation are still missing.

Table 1. *Age and dosage of methylphenidate of six subjects*

Subject	Age	Daily doses of methylphenidate
1	12.8y	15mg
2	11.8y	20mg
3	12.9y	80mg
4	13.0y	40mg
5	13.5y	54mg
6	14.8y	54mg

In a preliminary study we have recently addressed the effects of methylphenidate on the SWA topography. Six male adolescents with a mean age of 13.3 years and mean doses of 44mg per day (Table 1) were studied. Compared to age- and sex-matched healthy boys, the topography exhibited a lateral increase and a medial reduction of SWA. (Figure 1). Using single photon emission computerized tomography (SPECT) it was shown that abnormal brain activity was normalized by methylphenidate, such that abnormally increased activity in the somatosensory cortex was reduced while hypoactivity of the orbitofrontal cortex was increased to normal levels (Lee et al., 2005). Since the doses applied in the study were rather low, it could be speculated that the much larger amounts in our study potentiated

this effect leading to an overcorrection in medial areas. In addition, neurodevelopmental changes of ADHD may me masked by the high levels of methylphenidate.

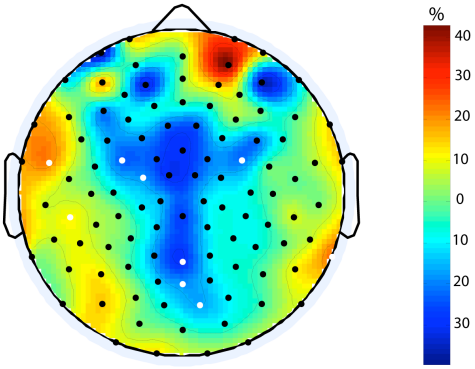

Figure 1. *Difference in topographical distribution of SWA (1-4.5 Hz) between six children with ADHD, treated with methylphenidate and six age- and sex matched healthy controls (ADHD minus Controls) based on the first hour of NREM sleep stages 2 and 3. Map was normalized across all electrodes for each individual and then averaged for each group. Values are colour coded (group differences in percentage). White dots indicate locations with significant differences (p<0.05, unpaired t-test).*

Impact of our findings on non-medication therapeutic interventions

Understanding of the mechanisms underlying a disorder is an important precondition for adequate therapeutic interventions. For example, theoretical models about the genesis of major depression have led to the development of new successful therapeutic strategies in the past, despite the fact that those models were never fully verified in empirical studies. Although the finding of a neuromaturational delay in children with ADHD may not be the exclusive cause, it still has important implications on the psychoeducation of caregivers: Parents and teachers may adapt their expectations once they understand that a child with ADHD reaches its limits of attention much faster, because its cognitive resources are not as mature as those of a healthy child of the same age. Reasonable adjustments of the daytime schedule, allowing for more

breaks and adapting the teaching to the possibilities of the child, may have positive effects, not only in terms of the current situation but also in terms of an adequate stimulation needed for proper development. In this respect also existing approaches, like reducing distraction by offering the child a seat at the front of the classroom, as well as advising parents to help the child to maintain a structured day, may be helpful interventions not only to reduce the symptoms but moreover help good interactions and support development (Döpfner et al., 2000). Finally, undisturbed sleep, adequate number of hours and regular bedtimes is essential for well being and proper daytime functioning (Short et al.), as well as the neuronal processes of brain development itself. Here dealing with the hyperactivity may be more important. Sufficient physical exercise during the day, reduction of excessive stimulation (e.g. computer games) (Higuchi et al., 2005) as well as meditation strategies for self-calmness may be helpful.

In summary, the effects of psychostimulants on neuronal development seem to be beneficial (Shaw et al., 2009). This may be the result of normalized brain activation (Lee et al., 2005) in part also due to improved activity-driven plasticity. However more studies addressing this issue need to follow. Also we should keep in mind that other long term effects, such as addictive behaviours or emotional disorders, which have been reported following treatment of methylphenidate in juvenile and adolescent rats (Bolanos et al., 2003; Carlezon et al., 2003) may not be reflected by changes in grey matter maturation. Apart from dosage, also the timing as well as genetic effects may modulate the outcome of psychostimulant treatment. Recently it was asked, how long-term plastic changes, induced by methylphenidate, could be best examined, because it is difficult to establish a rodent model for ADHD (Hyman, 2003). Because of the close relationship between SWA and synaptic changes, the investigation of SWA across the night and longitudinally in humans and animals may offer a marker of neuronal changes to understand effects of psychostimulants on brain plasticity.

Importance of this research

Finally the question needs to be answered: What are the advantages and the potential of this research? Two different possibilities of application are highlighted in the next sections.

Implications for a diagnostic tool

First of all, the investigation of SWA topography by means of hd-EEG may serve as a diagnostic tool in the future. The need for objective variables is demonstrated by the example of ADHD. Up to now the diagnosis of ADHD still depends to a large extent on subjective reports of patients, family members and caregivers. Additionally, many objective tests to estimate the degree of specific disturbances (like attention tests), are confounded with other factors such as motivation or daytime condition. The development of a reliable biological diagnostic test is therefore of great need. The application of hd-EEG to measure sleep SWA topography as a diagnostic tool would include several advances advantages: First, sleep is independent of motivational factors. Second, the measurement is objective, reliable and robust (highly replicable across nights). These factors are also of interest in study conditions, since many functional neuroimaging studies face similar problems, as diagnostic settings. Third, sleep SWA is a tool that is easy to analyse and not susceptible to moving artefacts, which is important for the investigation of children and patients. Fourth, it is cost-efficient and can be repeatedly applied without any ethical concerns. Finally, brain activity, as measured by SWA, may be more predictive for functional outcome than is grey matter volume.

However, there is an issue that will remain a challenge in the future: The application on the individual level as a diagnostic tool. There is a high variability and substantial overlap of measures of SWA between the compared groups. Comparison of mean SWA and standard deviation in the significant cluster electrodes between both groups showed 61.8% overlap (effect size: 0.6 (Cohen 1988)). The same result was found for a frontal/central ratio in SWA, an approximation for the state of maturation (Kurth et al., 2010a). This exacerbates the diagnostic use of this tool and makes predictions about behaviour or ability in an individual difficult. For example, although we have found

remarkable group differences between the SWA topography of children with and without ADHD, we cannot confidently tell from an individual topography if this child is affected by ADHD or not. Transferring predictions from group average maps to individual distributions of SWA is going to be a major challenge in future research. The search for a marker of brain maturation asks for longitudinal investigation of cortical development in a large sample. Monitoring the intraindividual changes of SWA may help establish reliable predictions about the trajectory and the actual state of cortical maturation.

Implications for research on psychiatric disorders

Second, the investigation of changes in SWA topography is also of great interest in psychiatry, because many disorders seem to have their origin already early in childhood or are developed during the change over phase in adolescence (Kessler et al., 2005). Since there does not seem to be a clear relationship between a certain genetic risk and one psychiatric phenotype (McClellan and King, 2010), the role of intermediate factors is increasingly important. Mapping the state of cortical maturation and the trajectory of brain development by hd-EEG maybe promising for several reasons:

First, investigation of differences of SWA in healthy subjects and those affected by a disorder may reveal the nature of the disorders. This was shown in our study in ADHD, uncovering a less mature pattern of SWA in children with ADHD, thereby pointing to brain development as contributing factor (Ringli et al., submitted-b). Thus, the study of extreme types of certain disorders, occurring early during development, like childhood-onset-schizophrenia (COS) or early forms of major depression in children may shed light into key factors responsible for the disorder.

Second, the longitudinal monitoring of changes in SWA may elucidate the timing of the disorder, because the trajectory of cortical maturation has been shown to be a better predictor for future outcome (Shaw et al., 2008). For example, the developmental trajectory of cortical thickness is more predictive of IQ than differences in adult cortical thickness (Shaw et al., 2006b). Also divergence of deviant grey matter trajectories in children with ADHD

towards typical development was related to remission of symptoms (Shaw et al., 2007).

Last, a recent study provides evidence, that SWA may not only be a useful tool to display the state of maturation, but even more seem to actively contribute to brain development (Kurth et al., submitted). Therefore, SWA may act as an intermediate factor in the development of a disorder. The understanding of how SWA contributes to normal and abnormal brain development may identify disturbed mechanisms underlying a disorder and offer potential sides of intervention.

Outlook on future research

Since we have only just begun to use hd-EEG in children to investigate the SWA topography, there is a huge field of possibilities opening up, to study the relationship between cortical plasticity and SWA during development. Indeed, many more studies are needed to establish SWA as a diagnostic tool that is suitable for clinical use on the single subject basis and to further understand what the differences in SWA topography between specific groups reveal.

First, populations with extensive use of certain functions or a special expertise represent unique possibility to study use-dependent plastic effects. For example, substantial cortical reorganisation somatosensory and auditory cortex was found in professional musicians (Elbert et al., 1995; Pantev et al., 1998). The difference to non-musicians was even larger the earlier the musicians started their musical education. This connection between practice and neuronal alterations offers the opportunity to study use-dependent long-term changes, which may also be reflected in the SWA topography. Doing so in children adds the dimension of brain maturation, which may offer a model to understand use-dependent as well as developmental differences of SWA topography in the same subject.

Second, adolescence seems to be a sensitive time to the development of several disorders (Kessler et al., 2005; Paus et al., 2008). Since SWA topography enables the mapping of brain maturation, the nature and trajectories of psychopathological disorders should be addressed in future studies. Besides ADHD,

changes in neurodevelopmental trajectories were also shown to occur in childhood-onset schizophrenia (COS). Accelerated grey matter loss during adolescence was reported in patients with COS (Gogtay, 2008), which was persistent in fronto-temporal areas whereas the increased rate of cortical thinning ceased in parietal cortex. The mapping of the distribution of SWA in adolescents with COS may therefore reveal a frontal decrease in SWA compared to healthy controls of the same age. The longitudinal mapping of the SWA topography could be used to investigate regional differences in speed of grey matter decrease. Since deviations in the trajectory were not only found in affected COS patients but were also less pronounced in their healthy siblings, mapping of SWA in groups that differ in terms of grey matter and behavioural symptoms (normal grey matter decrease/no symptoms of COS, deviant grey matter decrease/no symptoms of COS, deviant grey matter decrease/symptoms of COS) may offer a possibility of defining an endophenotype for diagnosis, as was suggest for imaging studies (Shaw et al., 2010).

Third, the influence of drug intervention, especially methylphenidate, on slow wave sleep and downscaling is not known so far. Animal models as well as examination of children with long term intake are urgently needed to estimate the potential risk and benefit of this therapy. An experimental setting, in which the age as well as the duration or dose of drug treatment is varied, may shed light into the effects on maturation. Differences in SWA between children starting intake at different ages at constant doses or same length of treatment would be due to different effects of medication during development. Whereas differences between children of the same age but with differences in doses and/or duration of medical treatment would be dosage dependent and help establish guidelines for future dosage prescriptions.

Finally, there is first evidence that SWA may actively contribute to functional brain maturation (Kurth et al., submitted). However, the role of SWA during development needs to be examined thoroughly in animal models. We have postulated that SWA may influence the balance of formation and elimination of synapses by the regulation of

downscaling (Ringli and Huber, 2011). However, how this is done in detail and what mechanisms, also during wakefulness, contribute to the time-course of the inverted U-shape of SWA during development, should be investigated by manipulating SWA.

In conclusion, this thesis shows the importance of sleep research for the understanding of cortical plasticity during normal brain development, as well as in groups with functional differences. It demonstrates the potential of applying SWA topography as a tool to study developmental disorders. Although, we still have limited understanding of the role of SWA during brain maturation, we can conclude that sleep is more than just a passive counterpart of wakefulness.

References

Aberg ND, Brywe KG, Isgaard J (2006) Aspects of growth hormone and insulin-like growth factor-I related to neuroprotection, regeneration, and functional plasticity in the adult brain. ScientificWorldJournal 6:53-80.

Aeschbach D, Cutler AJ, Ronda JM (2008) A role for non-rapid-eye-movement sleep homeostasis in perceptual learning. J Neurosci 28:2766-2772.

Aeschbach D, Cajochen C, Landolt H, Borbely AA (1996) Homeostatic sleep regulation in habitual short sleepers and long sleepers. American Journal of Physiology-Regulatory Integrative and Comparative Physiology 270:R41-R53.

Aserinsky E, Kleitman N (1953) Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. Science 118:273-274.

Attwell D, Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab 21:1133-1145.

Banks S, Dinges DF (2007) Behavioral and physiological consequences of sleep restriction. J Clin Sleep Med 3:519-528.

Bellina V, Huber R, Rosanova M, Mariotti M, Tononi G, Massimini M (2008) Cortical excitability and sleep homeostasis in humans: a TMS/hd-EEG study. Journal of Sleep Research 17:39-39.

Berger H (1929) Über das Elektroenkephalogramm des Menschen. . Archiv für Psychiatrie und Nervenkrankheiten 87:527-570.

Bes F, Schulz H, Navelet Y, Salzarulo P (1991) The distribution of slow-wave sleep across the night: a comparison for infants, children, and adults. Sleep 14:5-12.

Blake H, Gerard R (1937) Brain potentials during sleep. AMJ Physiol 119:692-703.

Bölsterli BK, Schmitt B, Bast T, Critelli H, Jenni OG, Huber R (2011) Impaired Sleep Downscaling in Encephalopathy with Status Epilepticus during Sleep (ESES). Clin Neurophysiol.

Borbely AA (1982) A two process model of sleep regulation. Hum Neurobiol 1:195-204.

Borbély AA, Achermann P (2005) Homeostasis of human sleep and models of sleep regulation. In: Principles and Practice of Sleep Medicine. (Kryger MH, Roth T, Dement WC, eds), pp 405-417. Philadelphia: W.B. Saunders.

Bourgeois JP, Rakic P (1993) Changes of Synaptic Density in the Primary Visual-Cortex of the Macaque Monkey from Fetal to Adult Stage. Journal of Neuroscience 13:2801-2820.

Braun AR, Balkin TJ, Wesenten NJ, Carson RE, Varga M, Baldwin P, Selbie S, Belenky G, Herscovitch P (1997) Regional cerebral blood flow throughout the sleep-wake cycle. An H2(15)O PET study. Brain 120 (Pt 7):1173-1197.

Brewer GJ, Boehler MD, Pearson RA, DeMaris AA, Ide AN, Wheeler BC (2009) Neuron network activity scales exponentially with synapse density. J Neural Eng 6:014001.

Buchmann A, Ringli M, Kurth S, Schaerer M, Geiger A, Jenni OG, Huber R (2010) EEG Sleep Slow-Wave Activity as a Mirror of Cortical Maturation. Cereb Cortex.

Burman DD, Bitan T, Booth JR (2008) Sex differences in neural processing of language among children. Neuropsychologia 46:1349-1362.

Bushey D, Tononi G, Cirelli C (2011) Sleep and Synaptic Homeostasis: Structural Evidence in Drosophila. Science 332:1576-1581.

Campbell IG, Feinberg I (2009) Longitudinal trajectories of non-rapid eye movement delta and theta EEG as indicators of adolescent brain maturation. Proc Natl Acad Sci U S A.

Chugani HT (1998) A critical period of brain development: studies of cerebral glucose utilization with PET. Prev Med 27:184-188.

Chugani HTP, Michael E. (1987) Positron Emission Tomography Study of Human Brain Functional Development. Ann Neurol 22:487-497.

Cirelli C, Tononi G (2004) Locus ceruleus control of state-dependent gene expression. J Neurosci 24:5410-5419.

Conners CK, Sitarenios G, Parker JD, Epstein JN (1998) Revision and restandardization of the Conners Teacher Rating Scale (CTRS-R): factor structure, reliability, and criterion validity. J Abnorm Child Psychol 26:279-291.

Cooley JW, Tukey JW (1965) An Algorithm for Machine Calculation of Complex Fourier Series. Mathematics of Computation 19:297-&.

Daw NW (1995) Visual Development. New York: Plenum Press.

DeFelipe J, Marco P, Fairén A, Jones EG (1997) Inhibitory synaptogenesis in mouse somatosensory cortex. Cereb Cortex 7:619-34.

Döpfner M, Frölich J, Lehmkuhl G (2000) Ratgeber Hyperkinetische Störungen. Göttingen: Hogrefe.

Drechsler R, Brandeis D, Foldenyi M, Imhof K, Steinhausen HC (2005) The course of neuropsychological functions in children with attention deficit hyperactivity disorder from late childhood to early adolescence. J Child Psychol Psychiatry 46:824-836.

Driver HS, Dijk DJ, Werth E, Biedermann K, Borbely AA (1996) Sleep and the sleep electroencephalogram across the menstrual cycle in young healthy women. J Clin Endocrinol Metab 81:728-735.

Elbert T, Pantev C, Wienbruch C, Rockstroh B, Taub E (1995) Increased Cortical Representation of the Fingers of the Left Hand in String Players. Science 270:305-307.

Esser SK, Hill SL, Tononi G (2007) Sleep homeostasis and cortical synchronization: I. Modeling the effects of synaptic strength on sleep slow waves. Sleep 30:1617-1630.

Faraguna U, Vyazovskiy VV, Nelson AB, Tononi G, Cirelli C (2008) A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. Journal of Neuroscience 28:4088-4095.

Faraone SV, Sergeant J, Gillberg C, Biederman J (2003) The worldwide prevalence of ADHD: is it an American condition? World Psychiatry 2:104-113.

Faraone SV, Biederman J, Morley CP, Spencer TJ (2008) Effect of stimulants on height and weight: A review of the literature. Journal of the American Academy of Child and Adolescent Psychiatry 47:994-1009.

Feinberg I (1982) Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? J Psychiatr Res 17:319-334.

Feinberg I, Floyd T (1979) Systematic trends across the night in human sleep cycles. Psychophysiology 16:283-291.

Feinberg I, Campbell IG (2010) Sleep EEG changes during adolescence: An index of a fundamental brain reorganization. Brain and Cognition 72:56-65.

Feinberg I, Higgins LM, Khaw WY, Campbell IG (2006) The adolescent decline of NREM delta, an indicator of brain maturation, is linked to age and sex but not to pubertal stage. Am J Physiol Regul Integr Comp Physiol 291:R1724-1729.

Fiala BA, Joyce JN, Greenough WT (1978) Environmental complexity modulates growth of granule cell dendrites in developing but not adult hippocampus of rats. Exp Neurol 59:372-383.

Finelli LA, Borbely AA, Achermann P (2001a) Functional topography of the human nonREM sleep electroencephalogram. Eur J Neurosci 13:2282-2290.

Finelli LA, Achermann P, Borbely AA (2001b) Individual 'fingerprints' in human sleep EEG topography. Neuropsychopharmacology 25:S57-62.

Finelli LA, Baumann H, Borbely AA, Achermann P (2000) Dual electroencephalogram markers of human sleep homeostasis: correlation between theta activity in waking and slow-wave activity in sleep. Neuroscience 101:523-529.

Frank MG, Morrissette R, Heller HC (1998) Effects of sleep deprivation in neonatal rats. Am J Physiol 275:R148-157.

Frank MG, Issa NP, Stryker MP (2001) Sleep enhances plasticity in the developing visual cortex. Neuron 30:275-287.

Gais S, Lucas B, Born J (2006) Sleep after learning aids memory recall. Learn Mem 13:259-262.

Galland BC, Tripp EG, Taylor BJ (2010) The sleep of children with attention deficit hyperactivity disorder on and off methylphenidate: a matched case-control study. J Sleep Res 19:366-373.

Galvan A (2010) Neural Plasticity of Development and Learning. Human Brain Mapping 31:879-890.

Gasser T, Jennen-Steinmetz C, Sroka L, Verleger R, Mocks J (1988) Development of the EEG of school-age children and adolescents. II. Topography. Electroencephalogr Clin Neurophysiol 69:100-109.

Geering BA, Achermann P, Eggimann F, Borbely AA (1993) Period-Amplitude Analysis and Power Spectral-Analysis - a Comparison Based on All-Night Sleep Eeg Recordings. Journal of Sleep Research 2:121-129.

Giedd JN (2004) Structural magnetic resonance imaging of the adolescent brain. Ann N Y Acad Sci 1021:77-85.

Giedd JN (2008) The teen brain: insights from neuroimaging. J Adolesc Health 42:335-343.

Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, Paus T, Evans AC, Rapoport JL (1999) Brain development during childhood and adolescence: a longitudinal MRI study. Nat Neurosci 2:861-863.

Giedke H, Schwarzler F (2002) Therapeutic use of sleep deprivation in depression. Sleep Med Rev 6:361-377.

Gogtay N (2008) Cortical brain development in schizophrenia: insights from neuroimaging studies in childhood-onset schizophrenia. Schizophr Bull 34:30-36.

Gombos F, Bodizs R, Kovacs I (2010) Atypical sleep architecture and altered EEG spectra in Williams syndrome. J Intellect Disabil Res.

Greenough WT, Volkmar FR, Juraska JM (1973) Effects of rearing complexity on dendritic branching in frontolateral and temporal cortex of the rat. Exp Neurol 41:371-378.

Guzman-Marin R, Suntsova N, Methippara M, Greiffenstein R, Szymusiak R, McGinty D (2005) Sleep deprivation suppresses neurogenesis in the adult hippocampus of rats. Eur J Neurosci 22:2111-2116.

Harasty J, Double KL, Halliday GM, Kril JJ, McRitchie DA (1997) Language-associated cortical regions are proportionally larger in the female brain. Arch Neurol 54:171-176.

Higuchi S, Motohashi Y, Liu Y, Maeda A (2005) Effects of playing a computer game using a bright display on presleep physiological variables,

sleep latency, slow wave sleep and REM sleep. J Sleep Res 14:267-273.

Hua JY, Smith SJ (2004) Neural activity and the dynamics of central nervous system development. Nat Neurosci 7:327-332.

Huber R, Tononi G, Cirelli C (2007a) Exploratory behavior, cortical BDNF expression, and sleep homeostasis. Sleep 30:129-139.

Huber R, Ghilardi MF, Massimini M, Tononi G (2004) Local sleep and learning. Nature 430:78-81.

Huber R, Esser SK, Ferrarelli F, Massimini M, Peterson MJ, Tononi G (2007b) TMS-induced cortical potentiation during wakefulness locally increases slow wave activity during sleep. PLoS One 2:e276.

Huber R, Ghilardi MF, Massimini M, Ferrarelli F, Riedner BA, Peterson MJ, Tononi G (2006) Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. Nat Neurosci 9:1169-1176.

Huber R, Graf T, Cote KA, Wittmann L, Gallmann E, Matter D, Schuderer J, Kuster N, Borbely AA, Achermann P (2000) Exposure to pulsed high-frequency electromagnetic field during waking affects human sleep EEG. Neuroreport 11:3321-3325.

Huttenlocher PR (1979) Synaptic density in human frontal cortex - developmental changes and effects of aging. Brain Res 163:195-205.

Huttenlocher PR, Dabholkar AS (1997) Regional differences in synaptogenesis in human cerebral cortex. J Comp Neurol 387:167-178.

Hutton C, Draganski B, Ashburner J, Weiskopf N (2009) A comparison between voxel-based cortical thickness and voxel-based morphometry in normal aging. Neuroimage 48:371-380.

Hyman SE (2003) Methylphenidate-induced plasticity: what should we be looking for? Biol Psychiatry 54:1310-1311.

Iber C, Ancoli-Israel S, Chesson AL, Quan SF (2007) The AASM Manual for the Scoring of Sleep and Associated Events. Westchester, IL: American Academy of Sleep Medicine.

Iglowstein I, Jenni OG, Molinari L, Largo RH (2003) Sleep duration from infancy to adolescence: reference values and generational trends. Pediatrics 111:302-307.

Innocenti GM, Price DJ (2005) Exuberance in the development of cortical networks. Nat Rev Neurosci 6:955-965.

Jancke L, Koeneke S, Hoppe A, Rominger C, Hanggi J (2009) The architecture of the golfer's brain. PLoS One 4:e4785.

Jenni OG, Carskadon MA (2004) Spectral analysis of the sleep electroencephalogram during adolescence. Sleep 27:774-783.

Jenni OG, Carskadon MA (2007) Sleep Behavior and Sleep Regulation from Infancy through Adolescence: Normative Aspects. Sleep Med Clin 2:321-329.

Jenni OG, Borbely AA, Achermann P (2004) Development of the nocturnal sleep electroencephalogram in human infants. Am J Physiol Regul Integr Comp Physiol 286:R528-538.

Jenni OG, van Reen E, Carskadon MA (2005) Regional differences of the sleep electroencephalogram in adolescents. J Sleep Res 14:141-147.

Jha SK, Jones BE, Coleman T, Steinmetz N, Law CT, Griffin G, Hawk J, Dabbish N, Kalatsky VA, Frank MG (2005) Sleep-dependent plasticity requires cortical activity. J Neurosci 25:9266-9274.

Johnson MH (2001) Functional brain development in humans. Nat Rev Neurosci 2:475-483.

Kattler H, Dijk DJ, Borbely AA (1994) Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. J Sleep Res 3:159-164.

Katz LC, Shatz CJ (1996) Synaptic activity and the construction of cortical circuits. Science 274:1133-1138.

Keshavan MS, Anderson S, Pettegrew JW (1994) Is schizophrenia due to excessive synaptic pruning in the prefrontal cortex? The Feinberg hypothesis revisited. J Psychiatr Res 28:239-265.

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005) Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62:593-602.

Kimura D (2000) Sex and Cognition. Cambridge, MA: MIT Press.

Knott GW, Quairiaux C, Genoud C, Welker E (2002) Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice. Neuron 34:265-273.

Knott GW, Holtmaat A, Wilbrecht L, Welker E, Svoboda K (2006) Spine growth precedes synapse formation in the adult neocortex in vivo. Nat Neurosci 9:1117-1124.

Kurth S, Ringli M, Geiger A, LeBourgeois M, Jenni OG, Huber R (2010a) Mapping of cortical activity in the first two decades of life: a high-density sleep electroencephalogram study. J Neurosci 30:13211-13219.

Kurth S, Jenni OG, Riedner BA, Tononi G, Carskadon MA, Huber R (2010b) Characteristics of sleep slow waves in children and adolescents. Sleep 33:475-480.

Kurth S, Ringli M, LeBourgeois M, Geiger A, Buchmann A, Jenni OG, Huber R (submitted) Cortical maturation is accompanied by increased sleep depth.

Kyle JG (1980) Auditory deprivation from birth--clarification of some issues. Br J Audiol 14:34-36.

Landolt HP, Dijk DJ, Gaus SE, Borbely AA (1995) Caffeine reduces low-frequency delta activity in the human sleep EEG. Neuropsychopharmacology 12:229-238.

Landsness EC, Crupi D, Hulse BK, Peterson MJ, Huber R, Ansari H, Coen M, Cirelli C, Benca RM, Ghilardi MF, Tononi G (2009) Sleep-dependent improvement in visuomotor learning: a causal role for slow waves. Sleep 32:1273-1284.

Lee JS, Kim BN, Kang E, Lee DS, Kim YK, Chung JK, Lee MC, Cho SC (2005) Regional cerebral blood flow in children with attention deficit hyperactivity disorder: comparison before and after methylphenidate treatment. Hum Brain Mapp 24:157-164.

Lendvai B, Stern EA, Chen B, Svoboda K (2000) Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex in vivo. Nature 404:876-881.

Leyfer OT, Woodruff-Borden J, Klein-Tasman BP, Fricke JS, Mervis CB (2006) Prevalence of psychiatric disorders in 4 to 16-year-olds with Williams syndrome. Am J Med Genet B Neuropsychiatr Genet 141B:615-622.

Liu ZW, Faraguna U, Cirelli C, Tononi G, Gao XB (2010) Direct evidence for wake-related increases and sleep-related decreases in synaptic strength in rodent cortex. J Neurosci 30:8671-8675.

Luders E, Gaser C, Narr KL, Toga AW (2009) Why sex matters: brain size independent differences in gray matter distributions between men and women. J Neurosci 29:14265-14270.

Luna B, Sweeney JA (2004) The emergence of collaborative brain function: FMRI studies of the development of response inhibition. Ann N Y Acad Sci 1021:296-309.

Maatta S, Landsness E, Sarasso S, Ferrarelli F, Ferreri F, Ghilardi MF, Tononi G (2010) The effects of morning training on night sleep: A behavioral and EEG study. Brain Res Bull.

Maret S, Faraguna U, Nelson AB, Cirelli C, Tononi G (2011) Sleep and waking modulate spine turnover in the adolescent mouse cortex. Nat Neurosci 14:1418-1420.

Marshall L, Helgadottir H, Molle M, Born J (2006) Boosting slow oscillations during sleep potentiates memory. Nature 444:610-613.

Mason TB, 2nd, Teoh L, Calabro K, Traylor J, Karamessinis L, Schultz B, Samuel J, Gallagher PR, Marcus CL (2008) Rapid eye movement latency in children and adolescents. Pediatr Neurol 39:162-169.

Massimini M, Huber R, Ferrarelli F, Hill S, Tononi G (2004) The sleep slow oscillation as a traveling wave. J Neurosci 24:6862-6870.

McClellan J, King MC (2010) Genetic heterogeneity in human disease. Cell 141:210-217.

McCormick DA, Pape HC (1990) Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. J Physiol 431:291-318.

Mejo SL (1992) Anterograde amnesia linked to benzodiazepines. Nurse Pract 17:44, 49-50.

Miyamoto H, Katagiri H, Hensch T (2003) Experience-dependent slow-wave sleep development. Nat Neurosci 6:553-554.

Moffat SD, Kennedy KM, Rodrigue KM, Raz N (2007) Extrahippocampal contributions to age differences in human spatial navigation. Cereb Cortex 17:1274-1282.

Moore DS, Johnson SP (2008) Mental rotation in human infants: a sex difference. Psychol Sci 19:1063-1066.

Morris CA, Mervis CB (2000) Williams syndrome and related disorders. In: Annual Review of Genomics and Human Genetics I, pp 461-484.

Moscovitch M, Nadel L, Winocur G, Gilboa A, Rosenbaum RS (2006) The cognitive neuroscience of remote episodic, semantic and spatial memory. Current Opinion in Neurobiology 16:179-190.

Moura SR, Mezzomo CL, Cielo CA (2009) Phonemic awareness stimulation and its effects regarding the variable gender. Pro Fono 21:51-56.

Munoz DP, Broughton JR, Goldring JE, Armstrong IT (1998) Age-related performance of human subjects on saccadic eye movement tasks. Exp Brain Res 121:391-400.

Needham A, Barrett T, Peterman K (2002) A pick-me-up for infants' exploratory skills: Early simulated experiences reaching for objects using 'sticky mittens' enhances young infants' object exploration skills. Infant Behavior & Development 25:279-295.

Neubauer BA, Gross S, Hahn A (2008) Epilepsy in childhood and adolescence. Dtsch Arztebl Int 105:319-327; quiz 327-318.

Nishino S, Mao J, Sampathkumaran R, Shelton J (1998) Increased dopaminergic transmission mediates the wake-promoting effects of CNS stimulants. Sleep Res Online 1:49-61.

Obal F, Jr., Alfoldi P, Cady AB, Johannsen L, Sary G, Krueger JM (1988) Growth hormone-releasing factor enhances sleep in rats and rabbits. Am J Physiol 255:R310-316.

Ohayon MM, Carskadon MA, Guilleminault C, Vitiello MV (2004) Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. Sleep 27:1255-1273.

Olcese U, Esser SK, Tononi G (2010) Sleep and synaptic renormalization: A computational study. J Neurophysiol.

Olfson M (1992) Diagnosing mental disorders in office-based pediatric practice. J Dev Behav Pediatr 13:363-365.

Olini N, Kurth S, Huber R (2010) A longitudinal study of sleep slow wave activity in juvenile rats. Journal of Sleep Research 19:1-378.

Ozcaliskan S, Goldin-Meadow S (2010) Sex differences in language first appear in gesture. Developmental Science 13:752-760.

Paavonen EJ, Raikkonen K, Lahti J, Komsi N, Heinonen K, Pesonen AK, Jarvenpaa AL, Strandberg T, Kajantie E, Porkka-Heiskanen T (2009) Short sleep duration and behavioral symptoms of attention-deficit/hyperactivity disorder in healthy 7- to 8-year-old children. Pediatrics 123:e857-864.

Pantev C, Oostenveld R, Engelien A, Ross B, Roberts LE, Hoke M (1998) Increased auditory cortical representation in musicians. Nature 392:811-814.

Paus T (2005) Mapping brain maturation and cognitive development during adolescence. Trends Cogn Sci 9:60-68.

Paus T, Keshavan M, Giedd JN (2008) OPINION Why do many psychiatric disorders emerge during adolescence? Nature Reviews Neuroscience 9:947-957.

Paus T, Collins DL, Evans AC, Leonard G, Pike B, Zijdenbos A (2001) Maturation of white matter in the human brain: a review of magnetic resonance studies. Brain Res Bull 54:255-266.

Pietrzak RH, Mollica CM, Maruff P, Snyder PJ (2006) Cognitive effects of immediate-release methylphenidate in children with attention-deficit/hyperactivity disorder. Neuroscience and Biobehavioral Reviews 30:1225-1245.

Rabinowicz T, Petetot JM, Khoury JC, de Courten-Myers GM (2009) Neocortical maturation during adolescence: change in neuronal soma dimension. Brain Cogn 69:328-336.

Rakic P, Bourgeois JP, Goldman-Rakic PS (1994) Synaptic development of the cerebral cortex: implications for learning, memory, and mental illness. Prog Brain Res 102:227-243.

Rall W (1967) Distinguishing theoretical synaptic potentials computed for different soma-dendritic distributions of synaptic input. J Neurophysiol 30:1138-1168.

Rechtschaffen A, Kales A (1968) A manual of standardized terminology , techniques and scoring system for sleep stages of human subjects.: Washington, DC: US Public Health Service, US Government Printing Office.

Reynolds CF, 3rd, Kupfer DJ, Thase ME, Frank E, Jarrett DB, Coble PA, Hoch CC, Buysse DJ, Simons AD, Houck PR (1990) Sleep, gender, and depression: an analysis of gender effects on the electroencephalographic sleep of 302 depressed outpatients. Biol Psychiatry 28:673-684.

Riedner BA, Vyazovskiy VV, Huber R, Massimini M, Esser S, Murphy M, Tononi G (2007) Sleep homeostasis and cortical synchronization: III. A high-density EEG study of sleep slow waves in humans. Sleep 30:1643-1657.

Ringli M, Huber R (2011) Developmental aspects of sleep slow waves: linking sleep, brain maturation and behavior. Prog Brain Res 193:63-82.

Ringli M, Kurth S, Jenni OG, Huber R (submitted-a) The sleep EEG topography in adolescents shows sex differences in language areas.

Ringli M, Kurth S, Geiger A, Jenni OG, Huber R (2009) Local Increase of Sleep SWA After Visuomotor Learning in Children. Neuropsychobiology 59:260-260.

Ringli M, Souissi S, Kurth S, Brandeis D, Jenni OG, Huber R (submitted-b) Topography of sleep slow wave activity in children with attention-deficit/hyperactivity disorder.

Robert JJ, Hoffmann RF, Emslie GJ, Hughes C, Rintelmann J, Moore J, Armitage R (2006) Sex and age differences in sleep macroarchitecture in childhood and adolescent depression. Sleep 29:351-358.

Roffwarg HP, Muzio JN, Dement WC (1966a) Ontogenetic development of the human sleep-dream cycle. Science 152:604-619.

Roffwarg HP, Muzio JN, Dement WC (1966b) Ontogenetic Development of Human Sleep-Dream Cycle. Science 152:604-&.

Rubia K, Halari R, Cubillo A, Smith AB, Mohammad AM, Brammer M, Taylor E (2011) Methylphenidate Normalizes Fronto-Striatal Underactivation During Interference Inhibition in Medication-Naive Boys with Attention-Deficit Hyperactivity Disorder. Neuropsychopharmacology 36:1575-1586.

Rueda MR, Rothbart MK, McCandliss BD, Saccomanno L, Posner MI (2005) Training, maturation, and genetic influences on the development of

executive attention. Proceedings of the National Academy of Sciences of the United States of America 102:14931-14936.

Sadeh A, Raviv A, Gruber R (2000) Sleep patterns and sleep disruptions in school-age children. Dev Psychol 36:291-301.

Sadeh A, Gruber R, Raviv A (2002) Sleep, neurobehavioral functioning, and behavior problems in school-age children. Child Dev 73:405-417.

Saugstad LF (1994) The maturational theory of brain development and cerebral excitability in the multifactorially inherited manic-depressive psychosis and schizophrenia. Int J Psychophysiol 18:189-203.

Savitz J, Solms M, Ramesar R (2006) The molecular genetics of cognition: dopamine, COMT and BDNF. Genes Brain Behav 5:311-328.

Shaw P, Gogtay N, Rapoport J (2010) Childhood psychiatric disorders as anomalies in neurodevelopmental trajectories. Hum Brain Mapp 31:917-925.

Shaw P, Sharp WS, Morrison M, Eckstrand K, Greenstein DK, Clasen LS, Evans AC, Rapoport JL (2009) Psychostimulant Treatment and the Developing Cortex in Attention Deficit Hyperactivity Disorder. American Journal of Psychiatry 166:58-63.

Shaw P, Lerch J, Greenstein D, Sharp W, Clasen L, Evans A, Giedd J, Castellanos FX, Rapoport J (2006a) Longitudinal mapping of cortical thickness and clinical outcome in children and adolescents with attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 63:540-549.

Shaw P, Greenstein D, Lerch J, Clasen L, Lenroot R, Gogtay N, Evans A, Rapoport J, Giedd J (2006b) Intellectual ability and cortical development in children and adolescents. Nature 440:676-679.

Shaw P, Eckstrand K, Sharp W, Blumenthal J, Lerch JP, Greenstein D, Clasen L, Evans A, Giedd J, Rapoport JL (2007) Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. Proc Natl Acad Sci U S A 104:19649-19654.

Shaw P, Kabani NJ, Lerch JP, Eckstrand K, Lenroot R, Gogtay N, Greenstein D, Clasen L, Evans A, Rapoport JL, Giedd JN, Wise SP (2008) Neurodevelopmental trajectories of the human cerebral cortex. J Neurosci 28:3586-3594.

Short MA, Gradisar M, Wright H, Lack LC, Dohnt H, Carskadon MA Time for bed: parent-set bedtimes associated with improved sleep and daytime functioning in adolescents. Sleep 34:797-800.

Sowell ER, Thompson PM, Leonard CM, Welcome SE, Kan E, Toga AW (2004) Longitudinal mapping of cortical thickness and brain growth in normal children. J Neurosci 24:8223-8231.

Sowell ER, Thompson PM, Rex D, Kornsand D, Tessner KD, Jernigan TL, Toga AW (2002) Mapping sulcal pattern asymmetry and local

cortical surface gray matter distribution in vivo: Maturation in perisylvian cortices. Cerebral Cortex 12:17-26.

Spencer TJ, Biederman J, Mick E (2007) Attention-deficit/hyperactivity disorder: diagnosis, lifespan, comorbidities, and neurobiology. Ambul Pediatr 7:73-81.

Stanley N (1996) The future of sleep staging. Human Psychopharmacology-Clinical and Experimental 11:253-256.

Steen RG, Ogg RJ, Reddick WE, Kingsley PB (1997) Age-related changes in the pediatric brain: quantitative MR evidence of maturational changes during adolescence. AJNR Am J Neuroradiol 18:819-828.

Steriade M, Amzica F (1994) Dynamic coupling among neocortical neurons during evoked and spontaneous spike-wave seizure activity. J Neurophysiol 72:2051-2069.

Steriade M, Timofeev I (2003) Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. Neuron 37:563-576.

Steriade M, McCormick DA, Sejnowski TJ (1993) Thalamocortical oscillations in the sleeping and aroused brain. Science 262:679-685.

Steriade M, Timofeev I, Grenier F (2001) Natural waking and sleep states: a view from inside neocortical neurons. J Neurophysiol 85:1969-1985.

Talamini LM, Nieuwenhuis IL, Takashima A, Jensen O (2008) Sleep directly following learning benefits consolidation of spatial associative memory. Learn Mem 15:233-237.

Tanner J (1962) Growth at adolescence. Oxford: Blackwell.

Tarokh L, Carskadon MA (2010) Developmental changes in the human sleep EEG during early adolescence. Sleep 33:801-809.

Tassinari C, Dravet C, Roger J (1977) ESES: encephalopathy related to electrical status epilepticus during slow sleep. In: Proceedings of the 9th Congress International Federation of EEG and Clinical Neurophysiology, pp 529-530. Amsterdam.

Tassinari C, Rubboli G, Volpi L, Billard C, Bureau M (2005) Electrical status epilepticus during slow sleep (ESES or CSWS) including acquired epileptic aplasia (Landau-Kleffner sindrome). In: Epileptic syndromes in infancy, childhood and adolescence (Roger J, Bureau M, Dravet C, Genton P, Tassinari C, Wolf P, eds), pp 295-314. Montrouge: John Libbey Eurotext.

Tau GZ, Peterson BS (2010) Normal development of brain circuits. Neuropsychopharmacology 35:147-168.

Taylor E, Chadwick O, Heptinstall E, Danckaerts M (1996) Hyperactivity and conduct problems as risk factors for adolescent development. J Am Acad Child Adolesc Psychiatry 35:1213-1226.

Teller DY (1981) The Development of Visual-Acuity in Human and Monkey Infants. Trends in Neurosciences 4:21-24.

Tessier CR, Broadie K (2009) Activity-dependent modulation of neural circuit synaptic connectivity. Front Mol Neurosci 2:8.

Tononi G, Cirelli C (2006) Sleep function and synaptic homeostasis. Sleep Med Rev 10:49-62.

Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K (2002) Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature 420:788-794.

Tucker AM, Dinges DF, Van Dongen HP (2007) Trait interindividual differences in the sleep physiology of healthy young adults. J Sleep Res 16:170-180.

Tzuriel D, Egozi G (2010) Gender differences in spatial ability of young children: the effects of training and processing strategies. Child Dev 81:1417-1430.

Van Der Werf YD, Van Der Helm E, Schoonheim MM, Ridderikhoff A, Van Someren EJ (2009) Learning by observation requires an early sleep window. Proc Natl Acad Sci U S A 106:18926-18930.

Von Economo C (1929) The cytoarchitectonics of the human cerebral cortex. London: Oxford Medical Publications.

Vyazovskiy VV, Riedner BA, Cirelli C, Tononi G (2007) Sleep homeostasis and cortical synchronization: II. A local field potential study of sleep slow waves in the rat. Sleep 30:1631-1642.

Vyazovskiy VV, Olcese U, Lazimy YM, Faraguna U, Esser SK, Williams JC, Cirelli C, Tononi G (2009) Cortical firing and sleep homeostasis. Neuron 63:865-878.

Webb WB, Agnew HW, Jr. (1971) Stage 4 sleep: influence of time course variables. Science 174:1354-1356.

Werth E, Achermann P, Dijk DJ, Borbely AA (1997) Spindle frequency activity in the sleep EEG: individual differences and topographic distribution. Electroencephalogr Clin Neurophysiol 103:535-542.

Whitford TJ, Rennie CJ, Grieve SM, Clark CR, Gordon E, Williams LM (2007) Brain maturation in adolescence: concurrent changes in neuroanatomy and neurophysiology. Hum Brain Mapp 28:228-237.

Wiesel TN, Hubel DH (1963) Effects of Visual Deprivation on Morphology and Physiology of Cells in Cats Lateral Geniculate Body. Journal of Neurophysiology 26:978-&.

Witelson SF, Glezer, II, Kigar DL (1995) Women have greater density of neurons in posterior temporal cortex. J Neurosci 15:3418-3428.

Wolfson AR, Carskadon MA (1998) Sleep schedules and daytime functioning in adolescents. Child Dev 69:875-887.

Woo TU, Crowell AL (2005) Targeting synapses and myelin in the prevention of schizophrenia. Schizophr Res 73:193-207.

Zito JM, Safer DJ, dosReis S, Gardner JF, Boles M, Lynch F (2000) Trends in the prescribing of psychotropic medications to preschoolers. Jama 283:1025-1030.

Zuo Y, Yang G, Kwon E, Gan WB (2005a) Long-term sensory deprivation prevents dendritic spine loss in primary somatosensory cortex. Nature 436:261-265.

Zuo Y, Lin A, Chang P, Gan WB (2005b) Development of long-term dendritic spine stability in diverse regions of cerebral cortex. Neuron 46:181-189.

List of publications

Papers

Buchmann, A., **Ringli, M.**, Kurth, S., Schaerer, M., Geiger, A., Jenni, O. G., Huber, R. (2011). *EEG Sleep Slow-Wave Activity as a Mirror of Cortical Maturation.* Cerebral Cortex, 21(3): 607-15.

Kurth, S., **Ringli, M.**, Geiger, A., LeBourgeois, M., Jenni, O. G., Huber, R. (2011). *Mapping of Cortical Activity in the First Two Decades of Life: A High-Density Sleep Electroencephalogram Study.* Journal of Neuroscience, 30(40): 13211-13219.

Geiger, A., Huber, R., Kurth, S., **Ringli, M.**, Jenni, O. G., Achermann, P. (2011). *The sleep EEG as a marker of intellectual ability in school age children.* Sleep, 34(2): 181-189.

Buchmann, A., Kurth, S., **Ringli, M.**, Geiger, A., Jenni, O.G., Huber, R. (2011). *Anatomical markers of sleep slow wave activity derived from structural magnetic resonance images.* Journal of Sleep Research, doi: 10.1111/j.1365-2869.2011.00916.

Ringli, M. and Huber, R. (2011). Developmental aspects of sleep slow waves: linking sleep, brain maturation and behaviour. Progress in Brain Research, 193:63-82.

Meyer, M., Elmer, S., **Ringli, M.**, Oechslin, M.S., Baumann, S., Jancke, L. (2011). Long-term exposure to music enhances the sensitivity of the auditory system in children. European Journal of Neuroscience: 34(5):755-65.

Geiger, A., Huber, R., Kurth, S., **Ringli, M.**, Achermann, P. and Jenni, O.G. (2011). *Sleep EEG topography and children's intellectual ability.* NeuroReport (accepted).

Kurth, S., **Ringli, M.**, Geiger, A., LeBourgeois, M., Jenni, O.G., Huber, R. (2011). *Cortical Maturation is accompanied by increased Sleep Depth*. (submitted)

Ringli, M., Kurth, S., Jenni, O.G., Huber, R. (2011). The sleep EEG topography in adolescents shows sex differences in language areas. (submitted)

Ringli, M., Souissi, S., Kurth, S., Brandeis, D., Jenni, O.G., Huber, R. (2011) Topography of sleep slow wave activity in children with attention-deficit/hyperactivity disorder. (submitted)

Abstracts

Ringli, M., Meyer, M., Baumann, S., Jancke, L. Effects of musical training on auditory mismatch negativitiy (MMN) in Suzuki children, Meeting of the International Neuropsychological Society (INS), Swiss Association of Neuropsychologists (SVNP) and German Association of Neuropsychology (GNP) in Zurich, CH, 2006

Ringli, M., Kurth, S., Jenni, O.G., Tononi, G. and Huber R. (2008). *High-density sleep EEG recordings in children and adolescents.* Journal of Sleep Research, 17: 130-130 19th Congress of the European Sleep Research Society (ESRS) in Glasgow, GB, 2008

Ringli, M., Kurth, S., Jenni, O.G., Tononi, G., Huber, R. *High-density sleep EEG recordings in children and adolescents.* Symposium of the Zurich Center for Integrative Human Physiology in Zurich, CH, 2008

Ringli, M., Kurth, S., Geiger, A., Jenni, O.G., Huber, R. Local increase of sleep SWA in prepubertal children and adolescents after visuomotor learning. Symposium of the Neuroscience Center Zurich (ZNZ) in Zurich, CH, 2009

Ringli, M., Kurth, S., Geiger, A., Jenni, O.G., Huber, R. (2009). Local Increase of Sleep SWA After Visuomotor Learning in Children. Neuropsychobiology 59(4): 260-260, 03/2009 Annual Conference of the Swiss Society of Sleep Research, Sleep Medicine and Chronobiology (SSSSC) and the Swiss Society of Biological Psychiatry (SSBP) in Berne, CH, 2009

Ringli, M., Kurth, S., Jenni, O.G., Huber, R. *Sleep EEG topography during development reveals sex differences.* Annual Meeting of the Swiss Society for Neuroscience (SSN) in Basel, CH, 2011

Ringli, M., Souissi, S., Kurth, S., Brandeis, D., Jenni, O.G., Huber, R. (2011). *Topography of sleep slow wave activity in children with attention deficit hyperactivity disorder.* Annual Retreat of the Children's Research Center (CRC) in Au/ZH, CH, 2011

Ringli, M., Souissi, S., Kurth, S., Brandeis, D., Jenni, O.G., Huber, R. (2011). *Topography of sleep slow wave activity in children with attention deficit hyperactivity disorder.* Joint Meeting of the Swiss Society of Sleep Research, Sleep Medicine and Chronobiology (SSSSC) and the Swiss Neurological Society (SNS) in St.Gallen, CH, 2011